PLOT-LEVEL TO LARGE-SCALE EFFECTS OF ECOLOGICAL DIVERSIFICATION AND ENVIRONMENT ON BIODIVERSITY

Dissertation

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SYNOPSIS

Humanity's desire to preserve current biodiversity for future generations requires sound understanding of how present-day biodiversity evolved in the past and how it is structured today. Evolutionary biology traditionally investigates how a single ancestor can give rise to enormous amounts of species, and ecology inquires species-environment interactions and how these lead to observed biodiversity patterns. For example, an evolutionary biologist, standing in a highly diverse alpine meadow, could ask how all these diverse flowering plants have evolved from a single ancestor. The ecologist, however, standing in the same meadow, would seek to identify the key environmental factors that determine which of the 250,000-400,000 flowering plant species make it into a diverse alpine meadow. In the light of on-going global change we realize that the ecologist and the evolutionary biologist have to talk to each other, that the two disciplines must better integrate to develop successful measures that protect today's biodiversity. In this thesis, I combined methodologies from both research fields in an attempt to further integrate ecological and evolutionary research. The influence of patterns in trait and niche evolution on the assembly of communities constituted my main research focus, which I investigated in Restionaceae (Poales) of the Cape Floristic Region in South Africa. In addition, I examined how differential ecological settings among continents influenced ecological diversification in the replicated radiation of the grass subfamily Danthonioideae.

In CHAPTER II, I investigated how reseeder-resprouter ratios of local Restionaceae assemblages change with environmental conditions in the Cape Floristic Region (CFR). While resprouter species generally survive fire and resprout from their rhizomes, reseeder species do not survive fire and have to regenerate from seeds. I designed a field sampling and tested with ordination and mixed effect models whether reseeder-resprouter ratios differ among contrasting habitats and climatic conditions. I found that the reseeder-resprouter ratio varies with climate and soil type, being highest in dry, seasonal climate and nutrient-poor soils.

However, reseeder-resprouter ratio does not differ among wet and dry habitats. My results are unexpected because they contradict results from other cape plant groups and suggest that generalities regarding the fire survival strategies in the CFR should be avoided.

In CHAPTER III, I focused on evolutionary patterns in the fire-survival strategies. The life history dichotomy of reseeding vs. resprouting has been proposed to explain the exceptional plant species richness of the CFR. I contrasted the patterns of trait-specific diversification and niche evolution in the replicated Restionaceae radiations of South Africa and Australia. The employed Bayesian approach inferred faster diversification rates for reseeders in South Africa, but not in Australia. Further, I showed that climatic niches evolved faster in reseeder than in resprouter lineages and that niche optima are different between the two strategies. The results indicate that rapid shifts between fire-survival strategies can promote speciation if the two strategies are separated ecologically and the landscape is heterogeneous.

In CHAPTER IV, I tested the hypothesis that the importance of competition in assembling species varies along an environmental harshness gradient. I developed an integrative ecophylogenetics framework that allows to evaluate how estimated phylogenetic structure of local assemblages varies along an environmental gradient and at the same time incorporates potentially confounding effects like phylogenetic scale and species pool composition. Results indicate that phylogenetic clustering in Restionaceae assemblages decreases with increasing drought stress, consistent with the hypothesis. In addition, analyses under the framework revealed that phylogenetic scale affects estimated phylogenetic structure of local assemblages more than does species pool composition. The integrative ecophylogenetics framework provides a flexible framework and, as such, is well suited to examine the generality of my findings in a range of organisms and ecosystems.

In CHAPTER V, I studied how inter-continental climate differences affected the occupancy of climatic niches in Danthonioideae. The Danthonioideae have radiated repeatedly on several continents and, therefore, are ideal to investigate

effects of available climatic conditions on ecological diversification. I demonstrated that danthonioid niches differ significantly among continents. This could partly be explained by niche truncation, where the full potential range of the lineage cannot be expressed due to the regional absence of suitable climates. Further, I propose that niches, during diversification, have shifted towards regionally available climatic space. Finally, evolution of climatic niches in Danthonioideae follows a punctuational pattern, and most niche shifts appear to be associated with the colonization of new continents.

Overall, the work presented in my thesis demonstrates that combining evolutionary and ecological concepts and methodologies furthers our understanding of the genesis and maintenance of present-day biodiversity. However, my research is only one further step towards the integration of ecology and evolutionary biology. Many have to follow to achieve the synthetic comprehension that is needed to successfully protect biodiversity for future generations.

ZUSAMMENFASSUNG

Das menschliche Bedürfnis, die heutige Biodiversität für kommende Generationen zu bewahren setz voraus, dass wir verstehen wie Biodiversität im Lauf der Evolution entstanden ist und wie sie heute strukturiert ist. Die Evolutionsbiologie befasst sich traditionell mit der Entstehung einer unglaublichen Anzahl an Arten aus einer einzigen Art, während die Ökologie untersucht, wie Arten mit ihrer Umwelt interagieren und wie diese Interaktionen zu beobachteten Biodiversitäts-Strukturen führten. Man stelle sich einen Evolutionsbiologen in einer artenreichen Alpwiese vor: er fragt sich, wie aus einer einzigen Pflanze die Vielfalt an Blütenpflanzen entstanden sein kann. Ein Ökologe in genau der gleichen Wiese dagegen, versucht die Umweltfaktoren zu identifizieren, die bestimmen, welche der insgesamt 250,000-400,000 Blütenpflanzen weltweit in genau dieser Wiese vorkommen. Im Licht des voranschreitenden globalen Wandels realisieren wir schnell, dass der Evolutionsbiologe und der Ökologe miteinander kommunizieren müssen und dass die beiden Disziplinen integral vernetzt werden müssen, um die Möglichkeit zu schaffen, die heutige Biodiversität zu schützen. In meiner Dissertation habe ich Methoden aus beiden Forschungsfeldern kombiniert, um die notwendige Vernetzung von Evolutionsbiologie und Ökologie voranzutreiben. Mein Schwerpunktthema, der Einfluss von Prozessen und Mustern in der Evolution ökologischer Nischen auf die Vergesellschaftung von Arten, untersuchte ich an den Südafrikanischen Restionaceae (Poales). Darüber hinaus erforschte ich, wie sich unterschiedlichen klimatischen Bedingungen auf verschiedenen Kontinenten auf die ökologische Diversifizierung einer Gras-Unterfamilie (Danthonioideae) auswirkten.

In KAPITEL II untersuchte ich, ob sich das Reseeder-Resprouter Verhältnis in Restionaceae-Gesellschaften der Kap-Region Südafrikas entlang von Umweltgradienten verändert. Resprouter-Arten überleben Feuer normalerweise und Teile des Wurzelsystems keimen („resprouten“) wieder. Reseeder sterben bei Feuer dagegen komplett ab und sind darauf angewiesen, aus Samen zu keimen (zu „reseeden“). Ich habe eine Datenerhebung geplant, die es mir erlaubte, mit

Hilfe von Ordinationsmethoden und mixed-effect Modellen zu testen, ob sich das Reseeder-Resprouter Verhältnissen in verschiedenen Habitaten und Umweltbedingungen unterscheidet. Das Verhältnis unterscheidet sich tatsächlich und ist in trockenem, saisonalem Klima und auf nährstoffarmem Boden am grössten. Meine Resultate sind etwas unerwartet, weil sie älteren Forschungsresultaten wiedersprechen. Die neuen Erkenntnisse deuten darauf hin, dass Verallgemeinerungen bezüglich umweltspezifischer Reseeder-Resprouter Verhältnisse von einzelnen auf alle Arten vermieden werden sollten.

KAPITEL III befasst sich mit evolutionären Mustern im Zusammenhang mit Reseeder- und Resprouter-Strategien im Umgang mit Feuer, die mit verantwortlich gemacht werden für die aussergewöhnlich hohe Biodiversität der Kap-Region. Ich untersuchte in den zwei unabhängigen Restionaceae Radiationen von Südafrika und Australien, ob sich die Art- und Nischen-Diversifikation in Reseedern und Resproutern unterscheidet. Ich fand heraus, dass die Artbildung in Reseedern schneller voranschreitet als in Resproutern, allerdings nur in Südafrika. Ausserdem konnte ich aufzeigen, dass Nischenevolution in Reseedern schneller vorangeht, und dass sich die beiden Strategien auf unterschiedliche klimatische Bedingungen spezialisieren. Meine Resultate deuten darauf hin, dass Feuer-Strategiewechsel zur Artbildung beitragen können, wenn sie begleitet sind von Veränderungen in der ökologischen Nische.

In KAPITEL IV überprüfte ich die Hypothese, dass der Einfluss von Konkurrenz im Vergesellschaftungs-Prozess von Pflanzen mit zunehmend härter werdenden Umweltbedingungen abnimmt. Ich habe ein integratives ökophylogenetisches Analyseverfahren entwickelt, das die phylogenetische Struktur von Pflanzengesellschaften entlang von Umweltgradienten abschätzt und gleichzeitig potentiell störende Einflussgrössen mit einbezieht. Diese störenden Variablen sind zum Beispiel phylogenetische Skala oder die Definition des Art-Pools. Die Resultate zeigen dass die Verwandtschaft von Arten in Restionaceae Gesellschaften mit zunehmendem Trockenstress abnimmt, was die Hypothese bestätigt. Zusätzlich zeigten die Untersuchungen, dass die phylogenetische Skala einen grösseren Einfluss auf Schätzungen der phylogenetischen Struktur von Gesellschaften ausübt, als die Zusammensetzung des zu Grunde liegenden Art-

Pools. Das integrative ökophylogenetisches Analyseverfahren ist sehr flexibel und gut geeignet, die Allgemeingültigkeit meiner Resultate an anderen Organismen und in anderen Ökosystemen zu testen.

In KAPITEL V untersuchte ich, wie sich unterschiedliche Klimabedingungen auf die Evolution ökologischer Nischen auswirkt. Da sich die Danthonioideae unabhängig auf mehreren Kontinenten evolutiv vervielfältigt haben, eignen sie sich gut, die Auswirkungen von verschiedenen Klimabedingungen auf ökologische Diversifikation zu studieren. Meine Forschung zeigt, dass sich die ökologischen Nischen der Danthonioideae zwischen den Kontinenten unterscheiden. Die Unterschiede können teilweise dadurch erklärt werden, dass für die Danthonioideae potentiell günstiges Klima auf gewissen Kontinenten gar nicht vorhanden ist. Weiter deuten die Resultate darauf hin, das sich die ökologischen Nischen im Lauf der Evolution auf jedem Kontinent unterschiedlich entwickelt haben, und zwar immer in Richtung der am Häufigsten vorkommenden klimatischen Bedingungen. Schliesslich geht die Nischen-Evolution in den Danthonioideae punktuell von statten, wobei die grössten Veränderungen im Zusammenhang mit der Kolonisierung von neuen Kontinenten aufzutreten scheinen.

Die Untersuchungen dieser Dissertation zeigen auf, dass die Kombination von ökologischen und evolutionsbiologischen Konzepten und Vorgehensweisen unser Verständnis der Entstehung und Erhaltung von Biodiversität fördert. Allerdings ist meine Forschung nur ein kleiner Schritt in Richtung eines synthetischen Verständnisses, das uns erlauben wird, die heutige Biodiversität für kommende Generationen zu erhalten.

CONTENT

CHAPTER I GENERAL INTRODUCTION

RAFAEL O. WÜEST

Integrating Ecology and Evolution

Evolutionary ecology is motivated by the inherent human fascination about how current biodiversity has evolved in the past, is structured today, and could be maintained in the future. In the light of on-going global change, the latter seems to gain priority over the others because biodiversity is valuable for humanity in various ways by providing resources for our cultural, economic, and environmental well-being. However, we need to acquire sound understanding of how large numbers of species arose from single ancestors, how ecological characteristics evolved and how species coexistence is regulated in local assemblages in order to successfully protect and conserve today's biodiversity for future generations. Thus, integrating evolutionary and ecological research is of fundamental importance in present times.

The recognition of the need to integrate ecology and evolutionary biology is exemplarily illustrated in the development of community ecology research. Since Diamond's (1975) notion of permissible and forbidden combinations of coexisting species, local assemblages were thought to be filtered from a species pool, following certain assembly rules (Keddy 1992, Zobel 1997, Weiher and Keddy 1999, Götzenberger et al. 2012). While the evolution of the pool's species and ecological diversity was left to evolutionary biologists, assessing patterns in local species assemblages and inferring assembly rules from these patterns was left to ecologists (explicitely stated by Weiher and Keddy 1999, pointed out by Cavender-Bares et al. 2009). Only relatively recently, the research field acknowledged the need for integrating community ecology and evolutionary biology. This integration is important because the relationships between coexistence patterns and evolutionary processes are presumably not unidirectional from the evolution of species pools to assembly processes, but also involve feedbacks from coexisting species to evolutionary processes (Cavender-Bares et al. 2009). It is the inclusion of such feedbacks that will lead to the development of a comprehensive framework that allows predicting responses of species and species assemblages to environmental change (Cavender-Bares et al. 2009, Lavergne et al. 2010, Mouquet et al. 2012), a major task of ecologists in the light of ongoing global change.

In my dissertation, I aimed to combine tools from ecology and evolutionary biology to further our understanding of how biodiversity evolved in the past, how it is structured today, and how this structure is maintained over time. To achieve this, I moved through various spatial and temporal scales, covering everything from small spatial scales at the plot level to the largest

possible, the global scale that incorporates millions of years of evolutionary history. Generally, I was interested in the following questions:

- How is biodiversity structured within and among local assemblages?
  I addressed this question in CHAPTER II and in APPENDIX I

- How do traits that are relevant in the assembly of species evolve on a phylogenetic tree?
  This question is addressed in CHAPTER III.

- How are species assembled into local assemblages along environmental gradients?
  Aspects of this question are addressed in CHAPTER IV.

- How does ecological diversity evolve within clades at large scales?
  I explore diverse aspects of this question in CHAPTER V and APPENDICES II-V

Study Organisms

The South African Restionaceae (Poales, Figure 1) present an ideal case to study the interaction of evolutionary and ecological processes in governing biodiversity patterns for a number of reasons. First, these plants inhabit the Cape Floristic Region (CFR), one of the most species rich biodiversity hotspots outside the tropics (Myers et al. 2000). The CFR exhibits high levels of endemism (69%; Goldblatt and Manning 2000), leading biodiversity to be highly structured (Taylor 1984, Simmons and Cowling 1996, Privett et al. 2001, Thuiller et al. 2007, Galley et al. 2009). Second, South African Restionaceae likely originated in the Australian part of the fragmenting Gondwana (Linder et al. 2003) build a monophyletic clade (Linder 2003; corroborated by an updated phylogeny, see CHAPTER III) that diversified into ca. 350 extant species (Linder 2003). The numerous species in a monophyletic clade are essential when assessing evolutionary patterns of species' environmental characteristics. A phylogenetic hypothesis of Restionaceae already existed at the start of the project for both South African (Eldenas and Linder 2000, Moline and Linder 2005, Hardy et al. 2008) and Australian species (Briggs et al. 2000, 2010) and was further developed to increase gene coverage and species-level resolution (see CHAPTER III).

Two additional reasons that argue for the South African Restionaceae as study case are based on the species' and regions' ecology. First, the ecologically diverse South African Restionaceae are taxonomically (Linder 1984, Linder et al. 1998, Linder and Hardy 2010) and morphologically (Linder 2011) well described, and their geographic distribution is well

a)

b)

Figure 1 Sandstone Fynbos dominated by Restionaceae on the northern slopes of Hexrivierberge with view towards the Warm Bokkeveld (a) and an illustration of the diversity in Restionaceae inflorescences (b).

documented thanks to previous research (e.g. Linder and Mann 1998, Linder 2001, Moline and Linder 2006) and an occurrence database compiled by H. Peter Linder based on herbarium specimens and his own collections (see CHAPTER II for details). This species specific occurrence information allows estimating species' ecological characteristics because, second, the climate in the CFR was relatively stable through time compared to the northern hemisphere (Markgraf et al. 1995, Sandel et al. 2011). This temporal stability of CFR climate likely leads to an equilibrium of species' ranges and the environment, in contrast to the northern hemisphere where today's range limits are highly influenced by glacial cycles (e.g. Svenning and Skov 2004, Normand et al. 2011, Van der Veken et al. 2012, Dullinger et al. 2012). The assumption that species distributions are in equilibrium with their environment is important when estimating ecological characteristics from species distribution data (e.g. Guisan and Zimmerman 2000, Guisan and Thuiller 2005, Pearman et al. 2008, Václavík and Meentemeyer 2012). The sum of these reasons make the South African Restionaceae an ideal plant group to jointly study evolutionary and ecological phenomena.

A global perspective is clearly needed when attempting to understand biodiversity patterns to successfully protect biodiversity from global change threat. While South African Restionaceae are ideal to study biodiversity patterns at a local and regional scale and also allow for intercontinental comparison between Southern Africa and Australia (Linder et al. 2003, see also CHAPTER III), another study system is needed to address global scale questions. The Danthonioideae (Poales) are particularly suited to study ecological diversification at the global scale because this grass subfamily is found on every continent (Figure 2; Linder et al. 2000, 2013). The predominantly perennial grasses occur in temperate climates (Linder et al. 2000, Linder and Barker 2005), and consist of 281 species in 17 genera (Linder et al. 2010). The Danthonioideae supposedly originated in Africa, where they thereby undergoing replicated radiations in the Southern hemisphere (Linder et al. in prep). These replicated radiations are ideal to analyze ecological diversification in distinct environments among continents.

Outline of the Thesis and Main Findings

Biodiversity Structure in Local Assemblages

The smallest scale at which vegetation scientists analyze biodiversity patterns is the plot level. The main question thereby: how is biodiversity structured within and among local assemblages and does the structure vary with environmental conditions? Species of local

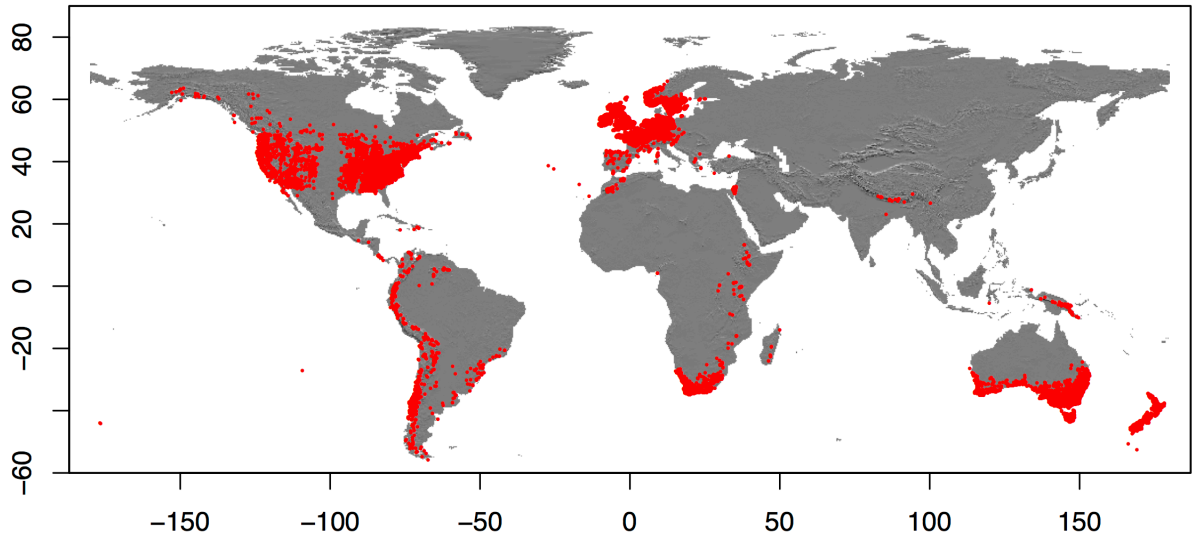

Figure 2 Global distribution of the grass subfamily Danthonioideae.

assemblages are selected of the species pool following a set of assembly rules (Keddy 1992, Zobel 1997, Weiher and Keddy 1999). These assembly rules (also seen as hierarchical sets of filters; Götzenberger et al. 2012) lead to biodiversity structure within and among local assemblages. Fire is doubtlessly an important filter that is shaping such structured biodiversity patterns in Mediterranean ecosystems like the CFR. Fire influences, for example, species richness (e.g. Guo 2001, Keeley et al. 2003), dominance patterns and composition (e.g. Bond et al. 2001, Schaffhauser et al. 2008, Vila-Cabrera et al. 2008), or phylogenetic structure (Verdú et al. 2007, Ojeda et al. 2010) of local assemblages. Thus, investigating fire-associated biodiversity patterns will increase our understanding of the local species assembly process in fire-prone ecosystems.

The effect of fire on Restionaceae assemblages in the CFR is likely mediated by a life history trait dichotomy, allowing two possibilities to cope with fire: the ability to resprout after fire (resprouters) or the need to germinate from seeds (reseeders). In **CHAPTER II** I assessed how reseeders and resprouters contribute to local assemblages of Restionaceae in the CFR. I designed a hierarchical sampling scheme to derive local assemblage data that ensures to cover the major climatic gradients and habitats in the CFR. The obtained plot data served to evaluate whether reseeder-resprouter ratio in local Restionaceae assemblages varies with climate, soil type, and local drainage conditions. Based on recent studies on various CFR taxa, I expected the reseeder-resprouter ratio to be highest in (i) mild mediterranean climates with short and mild summer drought (Ojeda et al. 2005), (ii) fertile soils (Power et al. 2011), and (iii) locally wet habitats (Rutherford et al. 2011).

The sampling, carried out in austral spring and summer of the years 2009-2001, yielded 235 plots that encompassed a total of 119 species, with a an average of 3.7 Restionaceae per plot. Using ordination techniques in combination with generalized linear mixed models, I could show that (i) the reseeder-resprouter ratio is higher in assemblages that experience dry and seasonal climate, and (ii) reseeder-resprouter ratio is higher on nutrient poor sandy soils, but (iii) does not differ between locally wet and dry habitats. The differential association of reseeders and resprouters to contrasting environmental conditions is expected (Ojeda et al. 2005, Power et al. 2011, Rutherford et al. 2011). However, the observed direction of environmental effects on reseeder-resprouter ratio often contradicted expectations. These contradictory results suggest that generalities regarding the fire survival strategies in the CFR should be avoided and that assemblage structure associated with this life history trait could be more variable than predicted so far.

Diversification and Evolution of Ecological Characteristics in Restionaceae

One aspect that could explain the observed structure in biodiversity patterns within and among local Restionaceae assemblages is differential associations of reseeders and resprouters to distinct environments (CHAPTER II), suggesting that reseeders and resprouters differ in their environmental niches. However, the results obtained in CHAPTER II do not support niche differentiation between reseeders and resprouters. This could be owed to the fact that, in CHAPTER II, I do not consider the evolutionary relationships between species. For instance, let's assume niche conservatism so that the entire South African Restionaceae clade will incorporate species that exhibit many diverse niches, but environmental niches of sister species will be similar, for example because they live in the same area with the same climate. Let's also assume that many sister species differ in the fire-response trait. In such a situation, a strong evolutionary differentiation between reseeders and resprouters, say, the shift in sister species from one particular fire response trait to the other is always associated with an adaptation of the environmental niche towards drier soil moisture conditions (Figure 3a), would not be detected without an evolutionary perspective added to the analysis. Both the reseeders and resprouters would have diverse environmental niches, but the grand means of reseeders and resprouters along the soil moisture gradient would roughly be the same (Figure 3b). This little example highlights the need for a study addressing this question in an evolutionary context.

In close collaboration with Glenn Litsios from the University of Lausanne, I compared the diversification between the two independent Restionaceae radiations in Southern Africa and

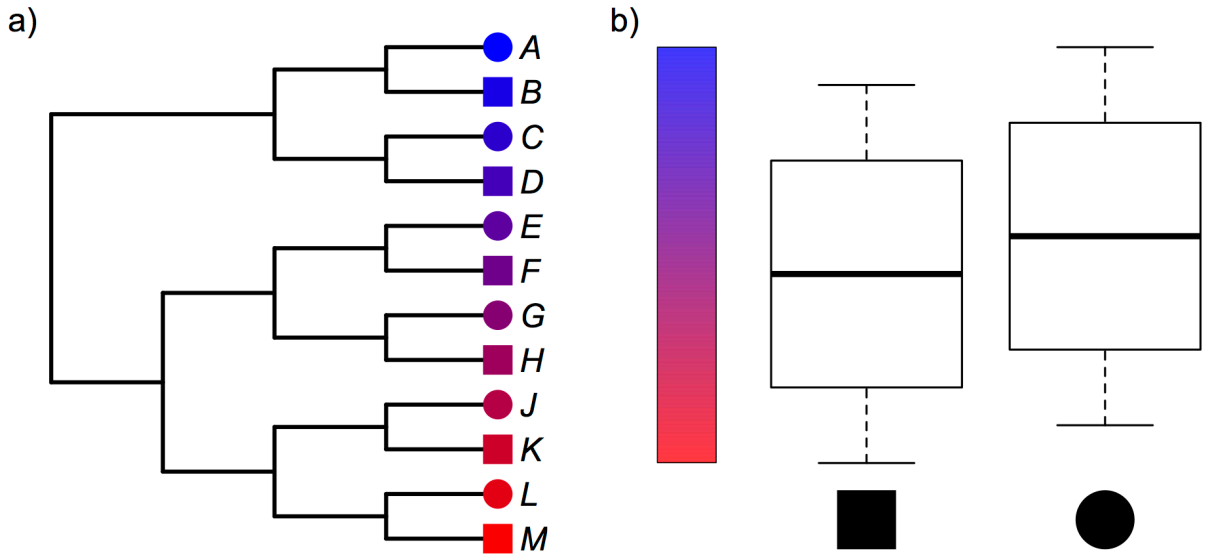

Figure 3 Sample phylogenetic tree (a) with reseeder (quadrats) and resprouter (circles) strategies that exhibit a highly labile phylogenetic pattern. The colors indicate preferred values of species A-M along a soil moisture gradient, ranging from dry (red) to wet (blue) conditions. Note that preferences along the moisture gradient exhibit phylogenetic signal, i.e. closely related species have similar soil moisture preferences. The boxplots (b) show that preferences in reseeders and resprouter along the soil moisture gradient are approximately the same if phylogenetic relationships are not taken into account.

Australia/New Zealand and associated evolutionary patterns in ecological characteristics in **CHAPTER III**. First, we investigated whether the life history dichotomy of reseeding versus resprouting Restionaceae is as evolutionary labile as in other taxa (Schnitzler et al. 2011, Gómez-González et al. 2011). Second, we tested whether diversification rates differed between the two strategies to cope with fire. This is expected based on recent research indicating that reseeders and resprouters could differ in terms of diversification rates because the two strategies to cope with fire differ in life history (phenology and generation time; Wells 1969, Linder 2003, Verdú et al. 2007). Third, we assessed whether reseeders and resprouters differed in their evolutionary trajectories, leading to fire-response-specific ecological preferences, as observed in various CFR taxa (Ojeda et al. 2005, Power et al. 2011, Rutherford et al. 2011).

A Bayesian approach to estimate diversification rates (BiSSE; Maddison et al. 2007) inferred higher diversification rates for reseeders than resprouters in South Africa, but not in Australia (Figure 2 in CHAPTER III). The different diversification rates could be linked to high environmental heterogeneity of the cape. Even though often employed as an important factor explaining the exceptional species richness of the CFR (Goldblatt 1978, Cowling et al. 1997, Linder 2003, Schnitzler et al. 2012), we could show for the first time that environmental heterogeneity is indeed higher in the CFR than in mediterranean regions of Australia and New

Zealand. Further, differential support for seven models of character evolution that contrasted the evolution of climatic preferences in reseeders and resprouters (using the OUwie framework; Beaulieu et al. 2012) indicated that evolutionary trajectories of ecological characteristics differ between reseeders and resprouters. The situation in Restionaceae indeed seems to be very similar to the example illustrated in Figure 3: speciation is mostly associated with a change in the fire-survival strategy. This change is associated with divergent trajectories in the adaptation of environmental niches, supposedly enhanced by steep environmental gradients in the CFR, as indicated by the high environmental heterogeneity. Ongoing climate change could affect the climate-driven fire regime of the CFR (Westerling et al. 2006, Wilson et al. 2010) and will likely favor the resprouters in the short term, as they are more persistent in changing environments (Bond and Midgley 2001, Vila-Cabrera et al. 2008, Cabral and Schurr 2010). In the long term, on the other hand, the shorter generation time of reseeders may allow them to counterbalance extinctions with adaptive speciation in newly available environmental conditions.

Assembly Rules Along Environmental Gradients

The importance of long-term historical and evolutionary processes in generating biodiversity patterns in local species assemblages was well recognized for a long time (e.g. MacArthur 1972, Ricklefs 1987). Still, the role of evolutionary processes often is neglected in studies that investigated the assembly of species into local assemblages (Weiher and Keddy 1999, Cavender-Bares et al. 2009). Community phylogenetics (Webb 2000, Webb et al. 2002, also termed ecophylogenetics; Mouquet et al. 2012) intends to close this research gap by assessing the phylogenetic structure of local assemblages. The phylogenetic structure of assemblages reflects the relatedness of coexisting species and is expected to vary with evolutionary patterns in traits (conserved vs. labile) and dominant assembly process (environmental filtering vs competitive exclusion; Webb et al. 2002). Therefore, the phylogenetic structure of local assemblages can be predicted if the evolutionary patterns of assembly-relevant traits and the dominant assembly process are known.

In **CHAPTER IV**, I extend the framework of ecophylogenetics to enable investigating the relative importance of competition and environmental filtering in determining the composition of local assemblages along an environmental gradient. Grime (1973, 1979) suggested that with increasing environmental harshness, competition is decreasingly important in determining the composition of local species assemblages. The phylogenetic structure of assemblages is, thus, also supposed to vary along a harshness gradient, because a

shift in the assembly process from competitive exclusion to environmental filtering changes the phylogenetic structure from clustered to overdispersed (or vice versa). Assembly processes are likely not shifting abruptly from one to the other and I, therefore, expect a rather smooth transition of phylogenetic structure along a harshness gradient. The direction and starting points of this transition will thereby depend on the evolutionary pattern in the relevant trait (Figure 4). I assess the phylogenetic structure of local assemblages along a moisture balance (MBAL) gradient in the CFR. MBAL is calculated as the ratio of potential evapotranspiration (PET) to precipitation (PREC). Consequently, high MBAL values (high PET and low PREC) indicate harsh environmental conditions due to drought stress, whereas low MBAL values (low PET and high PREC) reflect favorable conditions. I test whether the phylogenetic pattern in MBAL preferences in Restionaceae indicates conserved or convergent evolution to predict how the phylogenetic structure of local Restionaceae assemblages should vary along MBAL if Grime's hypothesis applies. I introduce an integrative ecophylogenetics framework that uses linear mixed effect models to incorporate potentially confounding effects like species pool composition, phylogenetic scale, and phylogenetic uncertainty. Therefore, I conducted the study at two phylogenetic scales to assess whether phylogenetic scale affects phylogenetic structure as expected (Slingsby and Verboom 2006, Cavender-Bares et al. 2006, Vamosi et al. 2009), included various species pool definitions because pool composition is known to influence measures of phylogenetic structure (Swenson et al. 2006, 2007, Kraft et al. 2007, Lessard et al. 2012), and repeated the analysis on a set of 100 trees to assess phylogenetic uncertainty.

I found that species specific preferences along the MBAL gradient exhibit a convergent phylogenetic pattern, leading to predict decreasing phylogenetic clustering along the MBAL gradient (dashed line in Figure 4). The results are consistent with the prediction as phylogenetic clustering decreases along the MBAL gradient in the CFR (Figure 3 in CHAPTER IV). This suggests that the importance of competition in assembling local Restionaceae assemblages indeed decreases with increasing environmental harshness, consistent with Grime's hypothesis(1973, 1979) and a growing number of studies that assess the phylogenetic structure of local assemblages along environmental gradients (Graham et al. 2009, Parra et al. 2010, Machac et al. 2011, González-Caro et al. 2012). The integrative ecophylogenetics framework revealed that phylogenetic scale had a bigger effect on estimated phylogenetic structure in Restionaceae assemblages than the composition of species pools. Furthermore, the framework indicates that regional processes start to gain primacy over local processes in assembling species at spatial scale of ca. 50x50km. Further research is needed

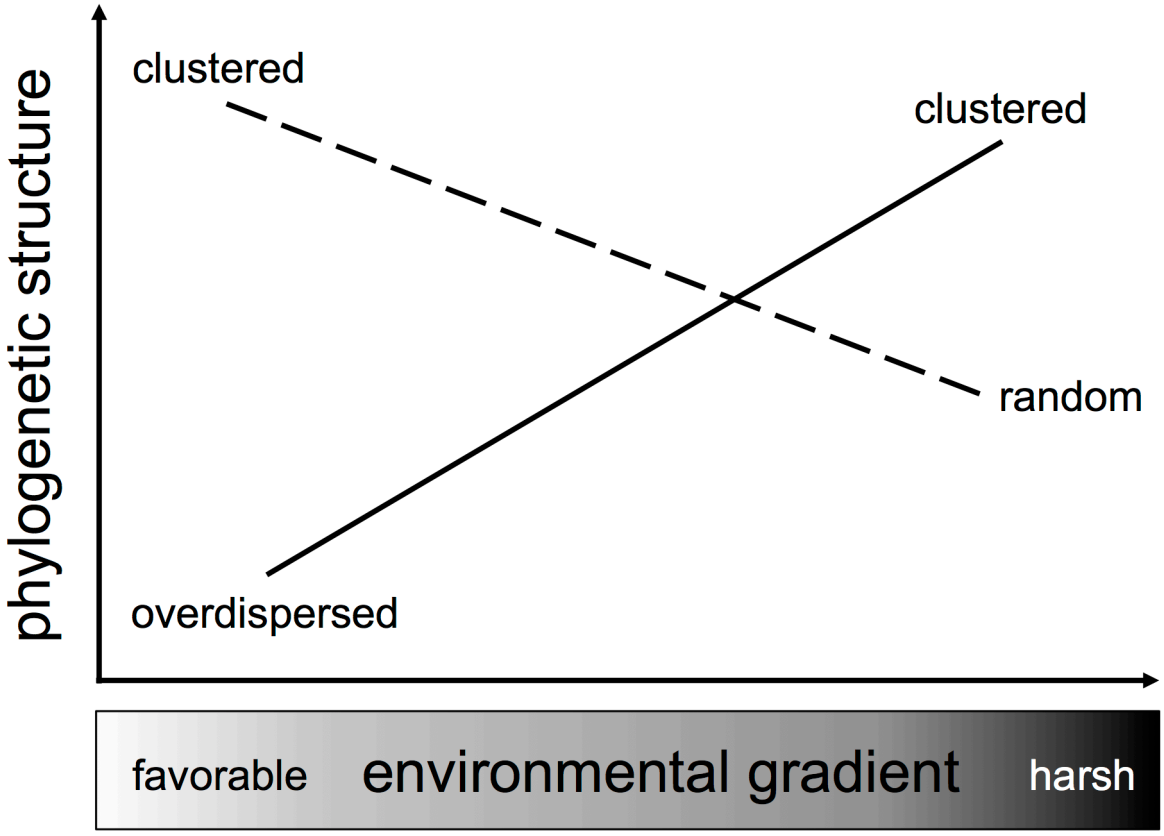

Figure 4 Scenarios how phylogenetic structure of local assemblages could vary along an environmental harshness gradient according to Grime's hypothesis. The solid line depicts the prediction assuming conserved evolution of traits, the dashed line convergent evolution, respectively.

that (i) incorporates additional environmental factors to better understand Restionaceae assembly and (ii) applies the integrative ecophylogenetics framework to a wide range of organisms and ecosystems in order to assess the generality of the presented findings.

Evolution of Ecological Diversity at Large Spatial Scales

Species diversification in Angiosperm lineages is influenced by intrinsic and extrinsic factors (Vamosi and Vamosi 2011). Intrinsic factors (e.g. key innovations, see APPENDIX III) are certainly important, but a growing number of studies suggests that extrinsic, i.e. ecological factors are of superior importance in determining angiosperm diversity (Ricklefs 2007, Rabosky 2009a, 2009b, Vamosi and Vamosi 2010, Wiens 2011). Analogously to species diversification, ecological diversification within a clade is also determined by intrinsic and extrinsic factors. Intrinsic constraints could be genetic (evolving new structures of physiologies), functional (ensuring the organism is still functional), or developmental (what is ontogenetically possible). Extrinsic factors, on the other hand, influence the evolution and expression of ecological diversity by defining the selective regime through the availability of

habitats or co-occurring species (Wiens et al. 2010). These extrinsic factors have been largely neglected so far in studying ecological diversification in angiosperm clades.

In **CHAPTER V** I compared how intrinsic and extrinsic factors interacted to influence the evolution of climatic niches in Danthonioideae among continents. I contrasted Brownian Motion (Felsenstein 1985) with Ohrnstein-Uhlenbeck evolutionary models (Butler and King 2004) to assess whether selective regimes differ between continents. I also tested whether these selective regimes can be predicted by available climatic conditions on the continents, which should be most facile if environmental traits are not constrained intrinsically. Intrinsic constraints in the evolution of niche traits are often subsumed under niche conservatism (NC), which describes that closely related species tend to have similar ecological niches (Wiens and Graham 2005, Wiens et al. 2010). I, therefore, assessed NC in the danthonioid grasses by means of phylogenetic signal (Pagel's λ Pagel 1997, 1999). Furthermore, I tested whether niche shifts in the evolution of danthonioid grasses were associated with intercontinental dispersal that would have led to punctuational evolution of niches using Pagel's κ (Pagel 1999). A better understanding of how the aforementioned processes interact improves our ability to interpret current niche occupancy, the evolutionary history, and future evolution of niches of globally distributed clades in a changing world.

The results indicated that the danthonioid niches differ between continents along the major climatic gradients, as identified by ordination. Further, I graphically demonstrated that danthonioid niches appear to be severely truncated on some continents, i.e. that some climatic conditions that danthonioid grasses occupy globally, are not present on the respective continents (Figure 2 in CHAPTER V). These intercontinental differences in available climatic conditions predicted inferred, continent-specific selective regimes to a large degree ($0.36<R2<0.94$, Table 2 in CHAPTER V). The predicted selective regimes thereby fitted inferred selective regimes best in cases where phylogenetic signal, i.e. intrinsic constraints, were lowest. Intercontinental differences in available climate possibly also explain the inferred pattern of punctual evolution if evolutionary niche shifts are associated with intercontinental dispersal. It is impossible to conclude from the correlative approaches I used in CHAPTER V what unexpressed potential the danthonioid grasses have to adapt to new environments, but knowing the range of possible ecological and evolutionary responses when confronted with altered climatic conditions helps to better predict how clades may respond to global change.

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CHAPTER II RESEEDER-RESPROUTER RATIO IN RESTIONACEAE VARIES WITH CLIMATE AND SOIL TYPE

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In review in PLoS ONE.

Fire influences plant biodiversity and community patterns in Mediterranean ecosystems. However, we know little about how evolutionary adaptations in life history that represent different strategies to cope with fire frequency and intensity are assembled in local communities. The Restionaceae (Poales) of the South African Cape Floristic Region have developed two major life history strategies to survive occurrences of fire. Reseeder species do not survive fire and regenerate from seeds, whereas resprouter species usually survive fire and resprout from belowground tissues, allowing the plant to recover from surviving remains. In this study we assess how resprouter-reseeder ratios of local Restionaceae assemblages change with environmental conditions in the Cape Floristic Region. We use ordination and generalized linear mixed models to investigate how the reseeder-resprouter ratio changes along climatic gradients and we test whether this ratio differs between contrasting habitat conditions. Our results show that the reseeder-resprouter ratio is affected by climate and edaphic conditions, but does not differ in contrasting soil drainage conditions. The reseeder-resprouter ratio is lower in favorable environmental conditions (aseasonal climate with high annual precipitation and fertile soils), which is unexpected given results from other Cape plant groups. These contradictory findings suggest that including the Restionaceae when studying the ecology and evolution of fire response traits in the Cape Floristic Region will provide a more complete picture of the diversity of observed patterns.

Introduction

The evolution and continued persistence of exceptional species richness in biodiversity hotspots are phenomena that continue to puzzle ecologists and evolutionary biologists. For example, the Cape Floristic Region (CFR) is one of the most species rich biodiversity hotspots outside of the tropics (Myers et al. 2000). Its high level of plant species endemism (69%; Goldblatt and Manning 2000) leads to high beta-diversity and turnover of species between local assemblages (Taylor 1984, Simmons and Cowling 1996). Fire is a factor that likely structures the high beta-diversity in the CFR and other mediterranean ecosystems (Bond and Van Wilgen 1996, Cowling et al. 1996, Tucker et al. 2012). However, the mechanisms responsible for high species diversity and turnover have not been explored in great detail.

Fire can affect biodiversity patterns in fire prone ecosystems in various ways. Species richness, for example, peaks two to three years after fire in coniferous forests of the Sierra Nevada (Keeley et al. 2003) and in the Californian Chaparral (Guo 2001), and subsequently declines with succession (Guo 2001). Differential fire intensity can force shifts in dominance patterns (Bond et al. 2001), and leads to an increase in phylogenetic clustering in local species assemblages (Verdu and Pausas 2007, Ojeda et al. 2010). Furthermore, long-term fire frequency can impact both local species richness and assemblage structure (Schaffhauser et al. 2008, Vila-Cabrera et al. 2008). These effects on assemblages can depend on a basic life history trait: the ability to resprout after fire (resprouters) or the need to germinate from seeds (reseeders; Clarke 2002, Lloret and Vila 2003, Pausas and Bradstock 2007). Variation in the structure of local assemblages could be linked to varying fire frequencies per se if the ability to resprout represents an adaptation to frequent fires (He et al. 2011) or be partially explained by other environmental factors if selective forces other than fire influence the evolution of this life history dichotomy (Hopper 2009). Despite the recognized importance of the reseeding-resprouting dichotomy to plant community structure (Bond and Midgley 2001), little is known about how reseeders and resprouters are distributed in local assemblages in the CFR.

Several studies of Cape plant clades provide the basis for empirical expectations for patterns of reseeder and resprouter compositions in local plant assemblages in the CFR. One expectation results from a simulation model for succession of *Erica* species, the largest plant genus in the CFR. Simulated reseeding populations of *Erica* replace resprouting populations only under conditions of short and mild summer drought (Ojeda et al. 2005). This suggests

higher reseeder-resprouter ratios should be found in local assemblages under favorable conditions (i.e. no intense summer drought). Additionally, reseeding legume genera in the CFR inhabit more fertile soils than resprouters (Power et al. 2011). These differences among legume genera suggest distinct edaphic niches for reseeders and resprouters. Further, observations of post-fire recovery in Restionaceae indicate that reseeders recover canopy cover faster in wet areas (Rutherford et al. 2011). Together, these results suggest that frequencies of reseeders and resprouters in CFR plant assemblages should vary with environmental conditions, with reseeders likely dominating under conditions of mild climate (i.e. moderate summer drought), fertile soils and abundant moisture.

In this study we assess how reseeders and resprouters contribute to local assemblages of Restionaceae species in the CFR of South Africa. We apply ordination and generalized linear mixed-effect models to investigate whether reseeder-resprouter ratios change with climatic conditions and among contrasting drainage conditions and soil types. We compare our findings to the literature-derived expectations outlined above and discuss possible explanations in an ecological context. The dichotomy of resprouting and reseeding potentially affects many ecological and evolutionary phenomena, ranging from competitive ability and its consequences on succession to fitness advantages due to regeneration trade-offs (Bond and Midgley 2001). Assessing how reseeders and resprouters are distributed in local assemblages is necessary to better understand these phenomena.

Methods

Ethics Statement

Field sampling permits were approved by Western Cape Nature Conservation Board for the Western Cape Province (Permit No. AAA005-00871) and the Department of Economic Development and Environmental Affairs for the Province of Eastern Cape (Permit No. CRO 124/10CR). We obtained permission from nature reserve managers and private land owners prior to the field sampling. No protected species were sampled. The data are available from ROW upon request.

Study System

We address the relationship between the resprouter-reseeder fire survival strategy and environmental conditions in assemblages of the South African Restionaceae (Poales). This family consists of ca. 450 species worldwide (Linder et al. 1998), with its greatest diversity in

the CFR (ca. 350 species (Linder 2003)). The South African Restionaceae diversification contributes to the exceptional plant diversity in the CFR, being here among the ten most species-rich clades (Linder 2003). The South African Restionaceae evolved great morphological and ecological diversity during their diversification, and are the dominant species in many Fynbos vegetation types (Mucina and Rutherford 2006). Our Restionaceae taxonomy follows Linder and Hardy (2010) for the Restioneae and Linder (1985) for the Willdenowieae.

Sampling Local Assemblages

We developed a hierarchical, stratified random scheme to sample Restionaceae assemblages along the major environmental gradients in the CFR. The first level of stratification was based on a classification of climatic variables, which we selected using principal component analysis (PCA) of climatic data associated with occurrences in a database of collections of Restionaceae species. The occurrence database (approximately 12,000 records) consists of geo-referenced herbarium specimens from the herbaria BOL and NBG, assembled by one of us (HPL). We extracted the entire set of bioclimatic variables available in the Worldclim dataset (Hijmans et al. 2005) at a resolution of 30 arc seconds using ArcGIS version 9.3 (ESRI Inc. 2009) and performed PCA in R version 2.13.2 (R Development Core Team 2009). We selected two of the original variables that had high loadings on the first two PCA axes, log-transformed annual precipitation and temperature seasonality (Table S1). For each of these variables, we separated values into 4 equal-interval categories and classified each pixel in the CFR. In addition to the climatic variables, we used the MODIS Active Fire data product (collection 3, years 2000-2008 (Davies et al. 2009)) to estimate time since most-recent fire as a third variable for stratification. To do this, we constructed three categories of time since last fire: burned within the previous 5 years, burned from five to eight years (the earliest MODIS data) and burned more than eight years previously. We excluded all areas that burned within the previous five years because of difficulties in identifying Restionaceae that are less than five years old. This exclusion is unlikely to bias our results because the mean fire return interval in natural areas of the CFR is 18.75 years (Wilson et al. 2010), with annual fire probability after five years being less than 0.1 historically and ca. 0.15 in recent years. The stratification scheme produced 29 strata (Figure 1).

We selected a minimal number of quarter degree squares (QDS, 0.25°x0.25°) for visitation in order to minimize transportation-effort (see Figure 1). Within each of the selected QDSs we excluded urban and agricultural areas, identified using the Environmental Potential Atlas for

South Africa (Van Riet et al. 1997), and then randomly selected 10-15 pixels (30x30 arc-seconds, ca. 1km^2) using the 'sample' function in R and an arbitrary seed number. The number of sampled pixels varied because each environmental stratum was sampled according to its frequency within a QDS. In the case that a chosen pixel only contained disturbed vegetation (settlement, grazing, agricultural fields, etc.), the pixel was replaced by a pixel of the same stratum, generally inside the QDS.

The second hierarchical level of the sampling scheme reflected the fact that the area corresponding to each of the selected pixels potentially contained various distinct habitats that could be occupied by particular Restionaceae species. To account for these habitat differences, we defined broad habitat categories based on soil type (sandy vs. loamy), slope (flat, medium slope, steep) and drainage (well-drained vs. seasonally wet). Every habitat type that occurred within a selected pixel was sampled with one 8x8m plot. In the field, we placed the sample plot within a habitat type to maximize the number of Restionaceae species present in the plot, while ensuring homogeneous habitat conditions. We recorded the Restionaceae species present in each sample plot.

Fire Survival Strategy

Data on the fire survival strategy was compiled by one of us (HPL) in an interactive key on the South African Restionaceae (Linder 2011), which lists the survival strategy for 232 of the 350 species (64%, strategy is unknown for the rest). We calculated for each of the sampled Restionaceae assemblages the reseeder-resprouter ratio, i.e. the ratio of reseeders to resprouters only including species with known fire survival strategies. Additionally, we calculated the reseeder-fraction, which represents the proportion of reseeders of all species present in an assemblage, regardless of whether the fire survival strategy is known. Comparison of results derived from both measures allowed us to assess potential bias stemming from incomplete knowledge of the fire response trait for all species.

Reseeders and resprouters in Australian Restionaceae exhibit clear morphological differences (Pate et al. 1991, Bell and Pate 1993, Meney and Pate 1999), but it is unclear whether these differences also apply to South African Restionaceae. Even if the differences are shared, they are difficult to diagnose in the field and do not allow diagnosis of a trait syndrome for the fire response strategy. The determination of fire survival trait states of South African Restionaceae, therefore, relied on existing field observations along fire boundaries, herbarium specimens, phytosociological observations, and literature comments (Linder 2011). The

determination of fire survival strategy, involving several methods of unequal difficulty in documenting the trait, could potentially bias trait determination and resulting frequencies. We performed a sensitivity analysis to assess how erroneous determination of fire survival strategy might influence our results. This analysis involved switching the fire survival trait of randomly selected species from reseeder to resprouter or vice versa. The fraction of randomly selected species ranged from 3% to 15% in 2% increments and random selection at each fraction was repeated 100 times. We repeated the statistical analyses on each of the resulting 700 altered-trait data sets and assessed whether the results changed qualitatively.

Statistical Analyses

We tested the effectiveness of our sampling scheme by comparing the sampled species richness at the QDS level (cumulative species richness of all plots within a QDS) with the expected species richness for the QDS that was obtained from the occurrence database. We used a Wilcoxon rank sum test to evaluate whether sampling effort differed between QDSs where sampled species richness exceeded expected species richness. Additionally, we assessed the species accumulation curve using tools implemented in the 'vegan' package (Oksanen et al. 2012) in R. The overall frequency distribution of reseeder-resprouter ratios in the sampled assemblages was assessed using histograms.

We applied the outlying mean index (OMI) ordination technique (Doledec et al. 2000) as implemented in the 'ade4' R package (Dray and Dufour 2007) in order to characterize the climatic conditions at the sample sites. We used the 19 bioclimatic variables from the Worldclim database (30x30 arc-seconds resolution; Hijmans et al. 2005) and potential evapotranspiration (PET) using the empirical equation of Jensen and Haise (1963), which was derived from monthly potential solar radiation layers created in GRASS GIS (GRASS Development Team 2010) and monthly mean temperature. The OMI was performed using species lists at the climate raster resolution ($1km^2$) because using the sample plots would lead to non-independence of the climate values. We subsequently projected the individual sample plots into the ordination space.

We used a generalized linear mixed model to assess whether the reseeder-resprouter ratio depended on environmental conditions. This allowed us to account for non-independence of the plots within each sampled pixel and to handle the number of reseeders and resprouters per sampled site as binomial response. The first two OMI axes, as well as drainage condition (dry vs. wet) and soil type (sandy vs. loamy) served as fixed effects. Pixel identity was specified as

random effect on the OMI axes. The model was fitted in R using the MCMCglmm package (Hadfield 2010). The MCMC chain was run for 5.5 million generations with a burn-in period of one million generations. The sampling interval of 3000 generations proved sufficient to avoid auto-correlated samples. We assessed the significance of the fixed effects by determining the 95% highest posterior density intervals (credible intervals, CI) of their estimates. A fixed effect was considered significant when the 95% CI did not include zero.

Results

Data are available for 235 plots sampled in austral spring and summer of the years 2009-2011 (Figure 1). We observe on average 3.7 Restionaceae species per plot (maximum of 12 species with a median of 3), resulting in 119 observed species. The majority of plots (70%) hold one to four species. The sampled species richness at QDS level is higher than the species richness expected from herbaria specimens in the majority of cases (58%, Figure 2). QDSs with sampled species richness below expectation (species richness derived from herbarium specimens) have significantly fewer sample sites (median of 2.5 vs. 10 sites, p=0.037). The species accumulation curve saturates to a large degree (Figure S1).

We only report the results based on reseeder-resprouter ratio derived from species with a known fire-survival trait because the two ways of calculating the proportion of reseeders per sampled assemblage (reseeder-resprouter ratio and reseeder-fraction) yield qualitatively similar results. The observed reseeder-resprouter ratios in the Restionaceae assemblages range from 0 to1 with a mean of 0.33 and a median of 0.25. Most sites either consist of only resprouters (40%) or only reseeders (12%), while the reseeder ratio for remaining sites is unimodally distributed around a mean of 0.43 (Figure S2).

The reseeder-resprouter ratio changes significantly with climatic conditions. The first two axes of the OMI analysis explain 80% of the total variation in the climate data (48% and 32% respectively). The major correlates of the first axis (hereafter referred to as OMI1) are annual precipitation (negatively correlated) and temperature seasonality (positively correlated, Figure 3 and Table S2) This results in aseasonal conditions with high precipitation at the lower end opposed to dry and seasonal conditions at the upper end of OMI1. The second axis (OMI2) represents a contrast between precipitation and temperature variables with hot and dry conditions at low values and cold and wet conditions at high values. This variability is geographically structured (Figure S3). The reseeder-resprouter ratio significantly increases

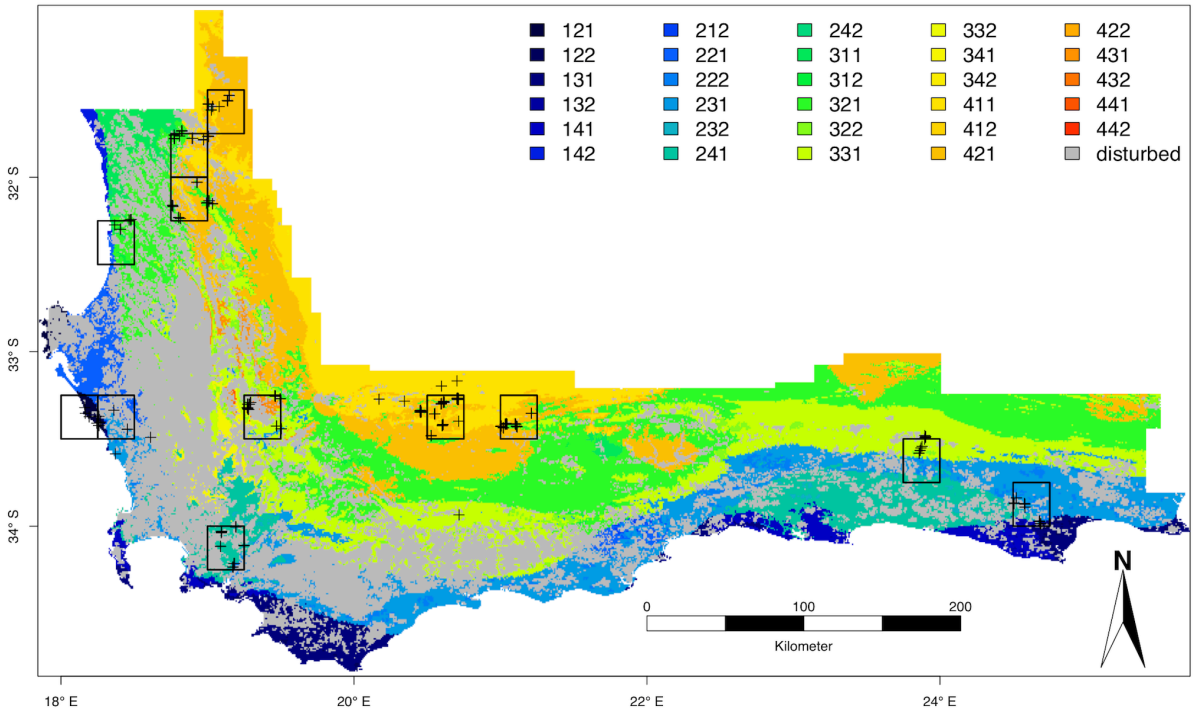

Figure 1 Sampling Scheme used to collect local Restionaceae assemblage data in the Cape Floristic Region. The background map indicates the environmental stratification with color coding shown in the legend. The grey areas refer to disturbed areas such as settlement or agriculture. The first digit in the legend corresponds to classes along Temperature range, the second digit classes along annual precipitation (log-transformed) and the third digit indicates classes of time since last fire. The squares represent the selected quarter degree squares and crosses indicated the sampled local assemblages.

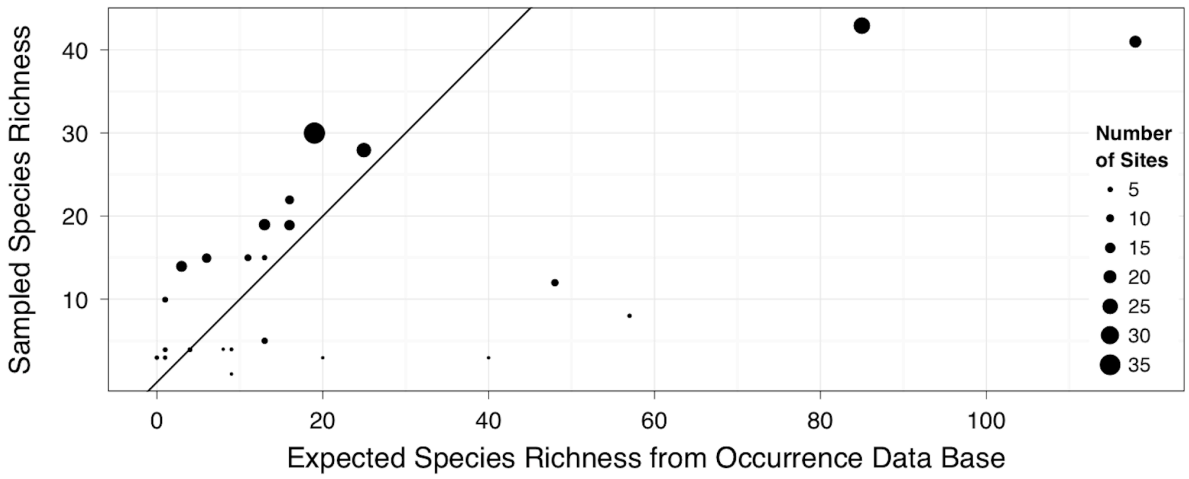

Figure 2. Expected and sampled Species richness at QDS level. Sampled species richness calculated as sum of every sample plot within one quarter degree square (QDS). Expected Species richness represents the number of species with known herbaria records within a QDS. The size of dots represents sampling effort, expressed as number of sites with a QDS (see legend), the line represents the 1:1 expectation.

along OMI1, as indicated by the positive slope estimate (posterior mean: 0.19) and the 95% CI of the GLMM slope estimate that excludes zero (Table 1). The 95% CI of the OMI2 estimates includes zero, indicating no evidence for change in reseeder-resprouter ratio along OMI2.

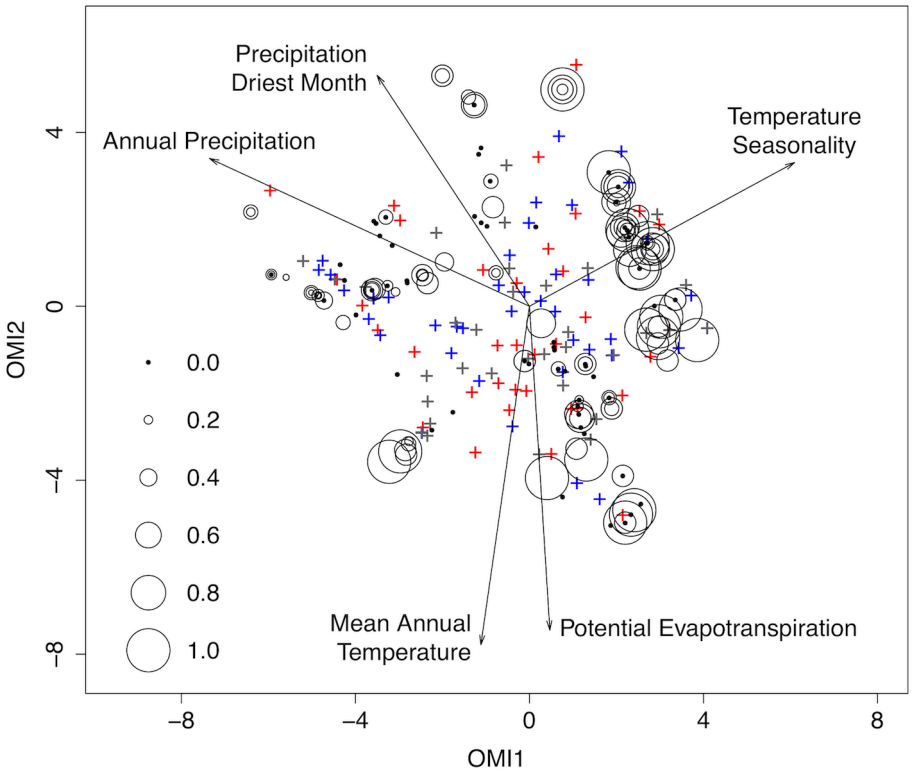

Figure 3 Outlying Mean Index (OMI) ordination results. The sizes of circles represent the reseeder ratios for each sample site as indicated in the legend. Crosses represent species' niche positions, with colors indicating fire survival strategy (red: reseeders; blue: resprouters; grey: unknown). The arrows represent correlations of the two ordination axes with the original variables (more details in Table S1).

Table 1 Results of the generalized linear mixed model. An estimate can be considered significant if the 95% credible interval (CI) does not include zero. OMI1 and OMI2 refer to the first and second axis of the outlying mean index ordination.

	posterior mean	**lower 95% CI**	**upper 95% CI**
Intercept (dry, loamy)	-1.39	-1.89	-0.94
OMI1	0.21	0.13	0.29
OMI2	0.02	-0.08	0.13
Drainage (wet)	0.13	-0.50	0.72
Soil Type (sandy)	0.73	0.23	1.24

We find that the reseeder-resprouter ratio is higher in sandy soils but does not differ among contrasting drainage conditions. The reseeder-resprouter ratio in wet conditions is on average 0.32 and does not differ significantly from the average (0.33) in dry habitats, as indicated by the GLMM results (Table 1, Figure 4). In contrast, the reseeder ratio is significantly higher on sandy soils (mean=0.36) than on loamy soils (mean=0.20; Table 1, Figure 4).

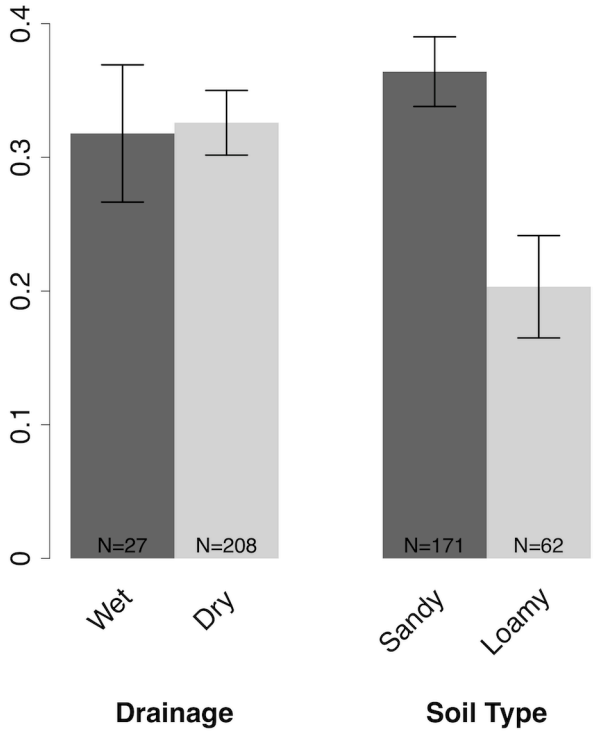

Figure 4 Reseeder ratios in different habitats. Mean±SE (error bars) of reseeder ratio in differing drainage conditions and soil types. N in each bar denotes the sample size in the respective group.

Sensitivity analysis revealed that our results are robust in regard to erroneous determination of the fire survival strategy. For each level of changed species assignment (3-15%) we obtain a distribution of test statistics due to the 100 random repetitions (Figure S4). There is no qualitative change in any of the analyses in the majority of the distribution (>50% of the random draws) if no more than 9% of the species' traits are recorded incorrectly. Analyses that delivered non-significant results using the original dataset (drainage and OMI2) are even more robust, as up to 15% of the recorded traits could be determined erroneously without changing the results qualitatively. Results vary depending on the analysis when considering the very restrictive 95% quantiles of the random draws distribution. OMI1 is significant up to

5% reassigned species traits, whereas, soil type could become non-significant already at 3% reassignment. Some analyses of OMI2 become significant starting from 3% change in assignment and similarly with soil drainage starting from 11%. Note that the 95% quantiles of the test statistics that exhibit high sensitivity may be overestimated because of outliers (Figure S4).

Discussion

We show that the reseeder-resprouter ratio changes with climate and edaphic conditions. Reseeder species tend to outnumber resprouters in dry and seasonal conditions, as well as on sandy soils that originate from sandstone bedrocks. Distinct climatic and edaphic niches of reseeders and resprouters could explain the observed pattern. Other factors such as the frequency and recurrence time of fire offer an alternative explanation.

Reseeder-Resprouter Ratio and Climatic Conditions

Our results show that reseeder-resprouter ratio in local assemblages increases with temperature seasonality and decreasing precipitation (Figure 3). The increasingly dry and seasonal conditions towards the upper end of the OMI1 gradient are mostly found in the rain shadow of the East-West and North-South mountain ranges that encounter intense summer droughts (Figure S3). Our findings contradict the expectation originating from a simulation model using *Erica* species (Ojeda et al. 2005), which predicts more reseeders in climates with mild Mediterranean summer droughts. However, reseeders in *Erica* are not capable of invading highly seasonal rainfall conditions (Ojeda et al. 2005). This is in line with the suggestion that unreliable rainfall and the resulting dry inter-rainfall periods lead to high mortality of seedlings and ultimately a higher extinction risk of seedling populations (Higgins et al. 2000, Cowling et al. 2005). Precipitation seasonality is indeed negatively correlated with OMI1 (-0.23, Table S2) and could partly explain the significant increase of reseeder ratio along OMI1. However, seasonal variation in total precipitation is unlikely the solitary driver of reseeder-resprouter ratio because precipitation seasonality is also highly correlated with OMI2, along which the reseeder ratio does not change significantly.

The observation that reseeder-resprouter ratio in Restionaceae assemblages changes along a climatic gradient suggests that species differ in their climatic niches. However, our results do not indicate differentiation of climatic niches between reseeders and resprouters (Figure 3). Differences in fire return interval could potentially explain absence of climatic niche

differentiation, because resprouters are likely more persistent in relatively fire-prone environments (Bond and Midgley 2001, Vila-Cabrera et al. 2008, Cabral and Schurr 2010; but see Thuiller et al. 2007). Further research is needed to explore whether plots with low reseeder ratio are located in environments with short fire return intervals, especially the large proportion of plots with no reseeders at all (42%). Shortest fire return intervals and, thus, lowest reseeder-resprouter ratio are expected in dry places with hot summers (Wilson et al. 2010), i.e. towards the upper end of the two OMI axes. However, this is contradictory to our findings (Table 1, Figure3). A thorough assessment of the influence of fire return intervals on reseeder-resprouter ratio is currently complicated by lack of a comprehensive model of fire return intervals for the entire CFR.

Reseeder-Resprouter Ratio and Habitat Conditions

Contrary to Rutherford et al. (2011), who found more reseeders in wet habitats, we find no significant difference in reseeder-resprouter ratio between wet and dry habitats (Table 1, Figure 4). This discrepancy is likely explained by the fact that Rutherford et al. investigated fynbos Restionaceae assemblages in the first three post-fire years, whereas our sampling excluded areas that burned five or fewer years prior to sampling. If reseeder regeneration were delayed (as reported for dry conditions; Rutherford et al. 2011), one would expect low reseeder ratios in early years and high reseeder ratios later on, which may explain the contrasting results. Rutherford et al. (2011) suggest a competitive advantage of resprouters over reseeders in fast regeneration as a possible explanation for the predominance of resprouters in early years. Results from comparisons of old and young fynbos communities support this idea, as the basal cover and number of large individuals of resprouter species were lower in old sites, suggesting successive erosion of resprouter dominance (Vlok and Yeaton 2000). Further research is needed to elucidate mechanisms behind this pattern.

We find a significantly higher reseeder ratio on sandy soils compared to loamy soils (Table 1, Figure 4). Loamy soils in the CFR are derived from shale and are more fertile than the sandy soils that stem from sandstone and quartzite (Cowling 1990). Thus, our results are inconsistent with findings for the Cape legumes, where reseeders in some genera consistently inhabit more fertile (here loamy) soils (Power et al. 2011). This suggests that reseeders and resprouters likely have distinct edaphic niches but associations between fire response strategies and soil fertility may vary among higher taxa. There may be additional factors that affect the differences in reseeder ratio between soil types because they differ in water retention potential, structure, and erosion patterns, due to their distinct grain sizes. Additional

studies are needed to link distinct soil differences with physiological properties of reseeders and resprouters because differing reseeder-resprouter ratios on different soils is alone inadequate to conclude that edaphic niches vary with these strategies in Restionaceae.

Fire Response Dichotomy

The observed pattern of reseeder-resprouter ratios in local Restionaceae assemblages is not necessarily driven by adaptation to fire. We observe that reseeders outnumber resprouters in dry and seasonal conditions (Figure 3). In dry conditions (low precipitation), plant germination is likely most successful if temperatures are not extremely hot, since hot temperatures may lead to soil-moisture deficiency due to high evaporative demand. If seed germination is synchronized with the seasonality (Lamont and Downes 2011), predictable temperature seasonality could ensure favorable germination conditions in reseeders (seeds germinate in the cold season with less evaporative demand). Resprouters, in contrast, may be less dependent on favorable conditions due to their better developed rhizomes (Pate et al. 1991). Even though this argumentation is in agreement with our observational data, experimental testing is needed to determine whether this trait dichotomy represents an adaptation to differential fire regimes, differences in environmental conditions, or a combination of the two.

The dichotomous life history trait of reseeders and resprouters is often assumed exclusive (e.g. Bell and Ojeda 1999, Lloret et al. 2003, Pausas and Bradstock 2007, Power et al. 2011). However, an exclusive dichotomy may not always apply because some species could have both resprouting and reseeding populations (Ojeda et al. 2005, Verdaguer and Ojeda 2005). Restionaceae species with both reseeder and resprouter individuals are documented in many South African genera (Linder 2011) but it is unclear whether the variation depends on fire intensity (Moreno and Oechel 1991, 1993), is an artifact of incorrect identification (of species or the trait) or represents among-population differentiation. The latter is supported by the closely related Australian genus *Lyginia* (Anarthriaceae) that encompasses both survival strategies in one species (*Lyginia barbata* (Bell and Pate 1993)), however, these variants are now described as distinct species (Briggs and Johnson 2000). Further research is needed to investigate whether certain South African Restionaceae species comprise both reseeding and resprouting populations, which would allow the evaluation of whether the pattern we find at the species level also holds at the population level. Intraspecific variation in the fire response trait and the fact that this trait is difficult to determine in the Restionaceae (Linder 2011) may confound the results of our study. Nonetheless, our sensitivity analysis demonstrates that the

results are robust to erroneous determination of this life history trait or misidentification of species. Up to 9% of the species or species' traits (depending on the threshold criterion, see Figure S4) could be changed without qualitatively affecting the results.

Sampling Scheme

We demonstrate that our sampling scheme allowed us to effectively sample a large number of the Restionaceae species present in the CFR. The number of species sampled at the level of QDSs exceeds the numbers expected based on herbarium collections when sampling effort is substantial (Figure 2). Our results suggest that sampling a QDS less than ten times is not sufficient to reach expected species richness. There are two QDSs that were sampled many times but did not reach expected species richness, likely because they are extremely species rich, i.e. represent the top 5% of QDSs in terms of species richness. Even though we only sampled one third of the 350 South African Restionaceae species, the species accumulation curve levels off to a large degree (Figure S1). The fact that it does not level off completely is not unexpected given the large number of narrowly distributed endemics in the CFR Restionaceae (Cowling and Holmes 1992, Linder 2001). Taken together, we conclude that our sampling design that ensures the compilation of broad climatic gradients as well as variation in local habitats allows the assessment of community patterns at the scale of the entire CFR.

Conclusions

We show that the reseeder-resprouter ratio is influenced by climate and edaphic conditions. This is consistent with our expectations because a number of recent studies have shown differential association of reseeders and resprouters to contrasting environmental conditions. However, the reseeder-resprouter ratio of the Restionaceae often contradicts the expected direction of the effects. We find higher reseeder ratios in dry and seasonal climates, which is inconsistent with results from *Erica* succession simulations (Ojeda et al. 2005), and lower reseeder ratios in fertile soils, which conflicts with the findings in legumes (Power et al. 2011). Given these contradictory results, we suggest that generalities regarding the fire survival strategies in the CFR should be avoided and that this trait might be more variable between different taxa than initially predicted. As a main component in many CFR vegetation types, Restionaceae offers promising opportunities for studying the dichotomy of the fire response trait, and help us to understand the ecology and evolution of the reseeding and resprouting strategy in the species rich Cape Floristic Region.

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Supporting Information

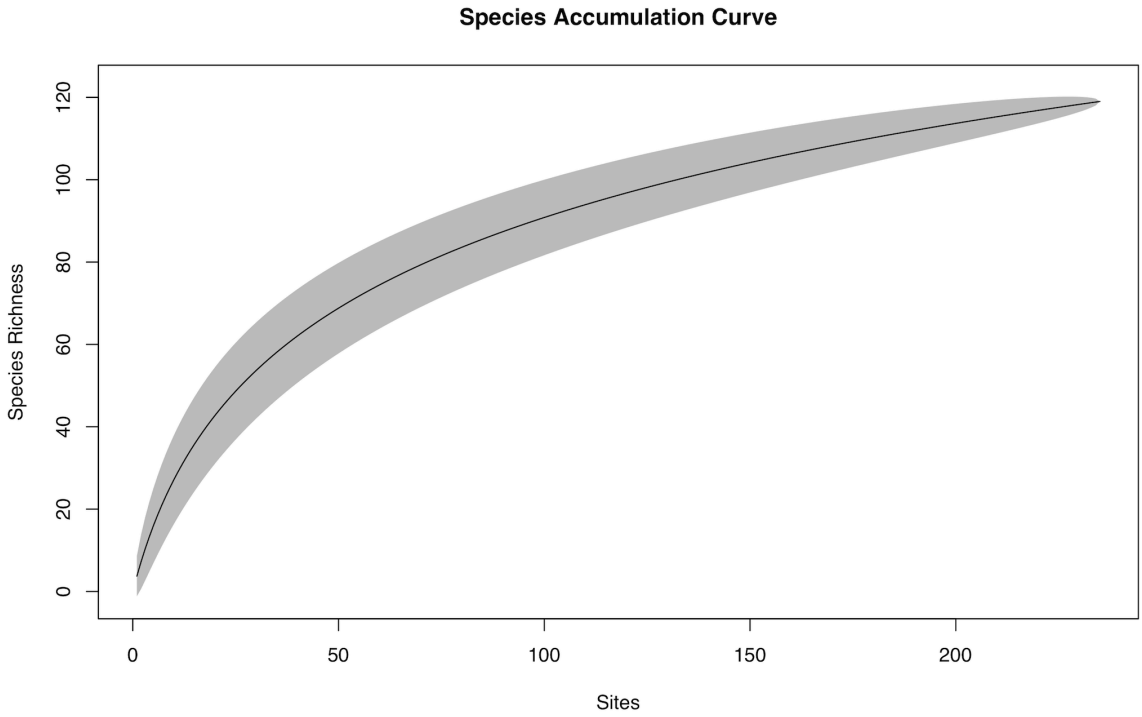

Figure S1 Species accumulation curve. The black line indicates the mean, the grey area the standard deviation derived from 100 random iterations.

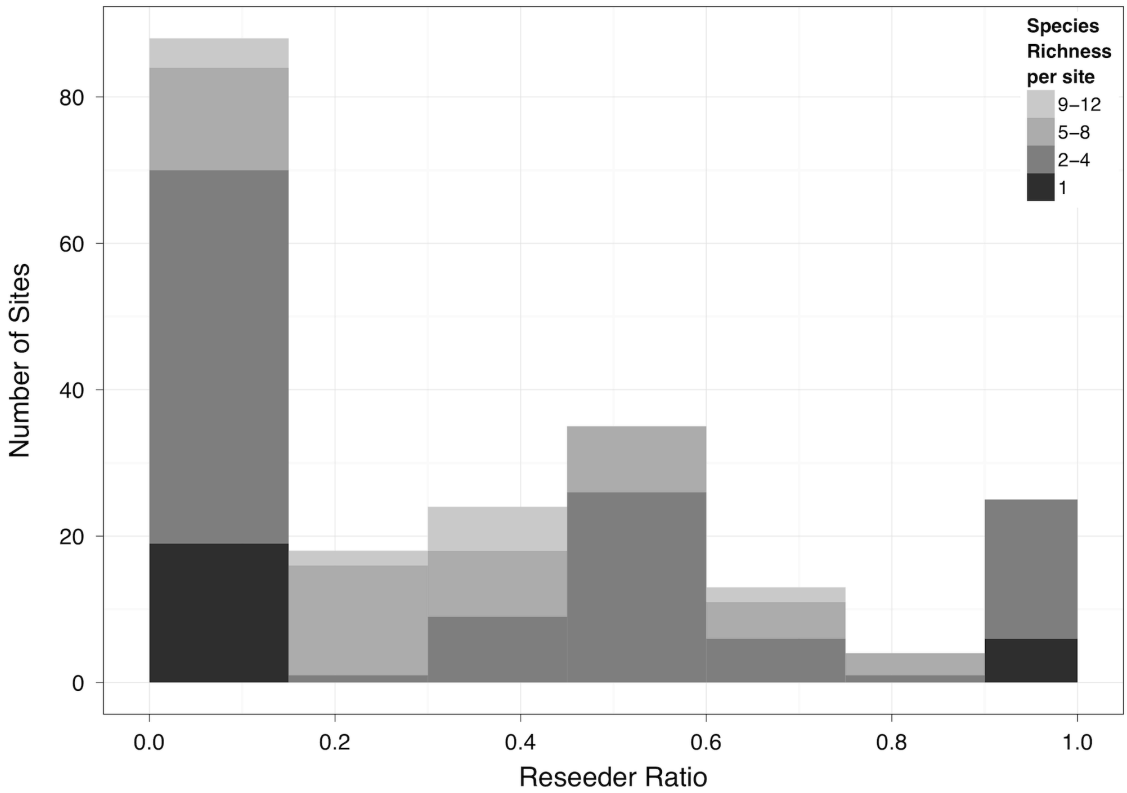

Figure S2 Histogram of reseeder ratios in samples. The grey shadings of the stacked bars represent the number of species in the sample sites (see legend).

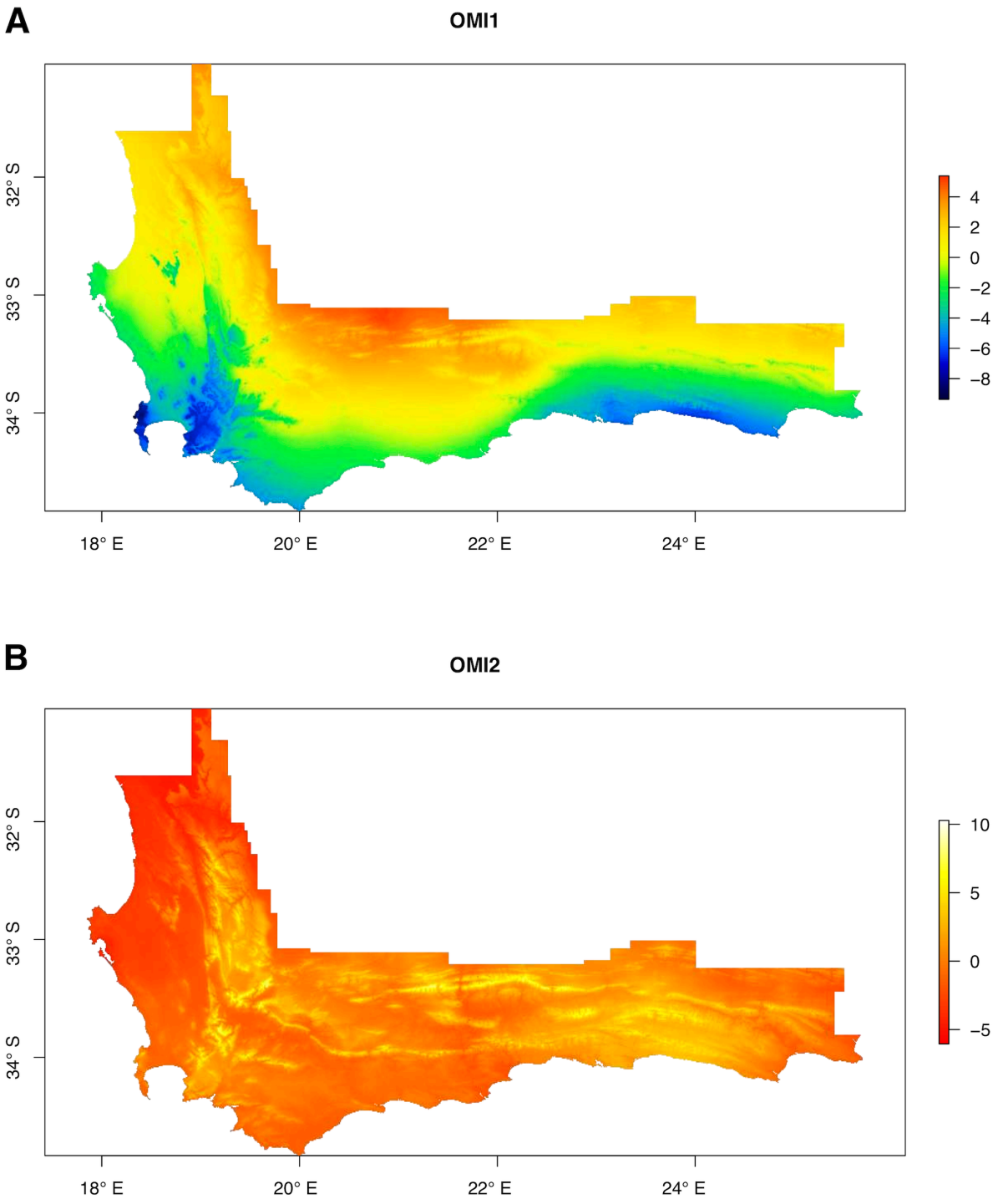

Figure S3 Spatial extrapolation of the first two axes of the outlying mean index ordination. The 19 bioclimatic variables were used to transform each pixel into the ordination space. Represented are axes one (OMI1) and two (OMI2), with colors coding for the scoring on the axes according to the color legends.

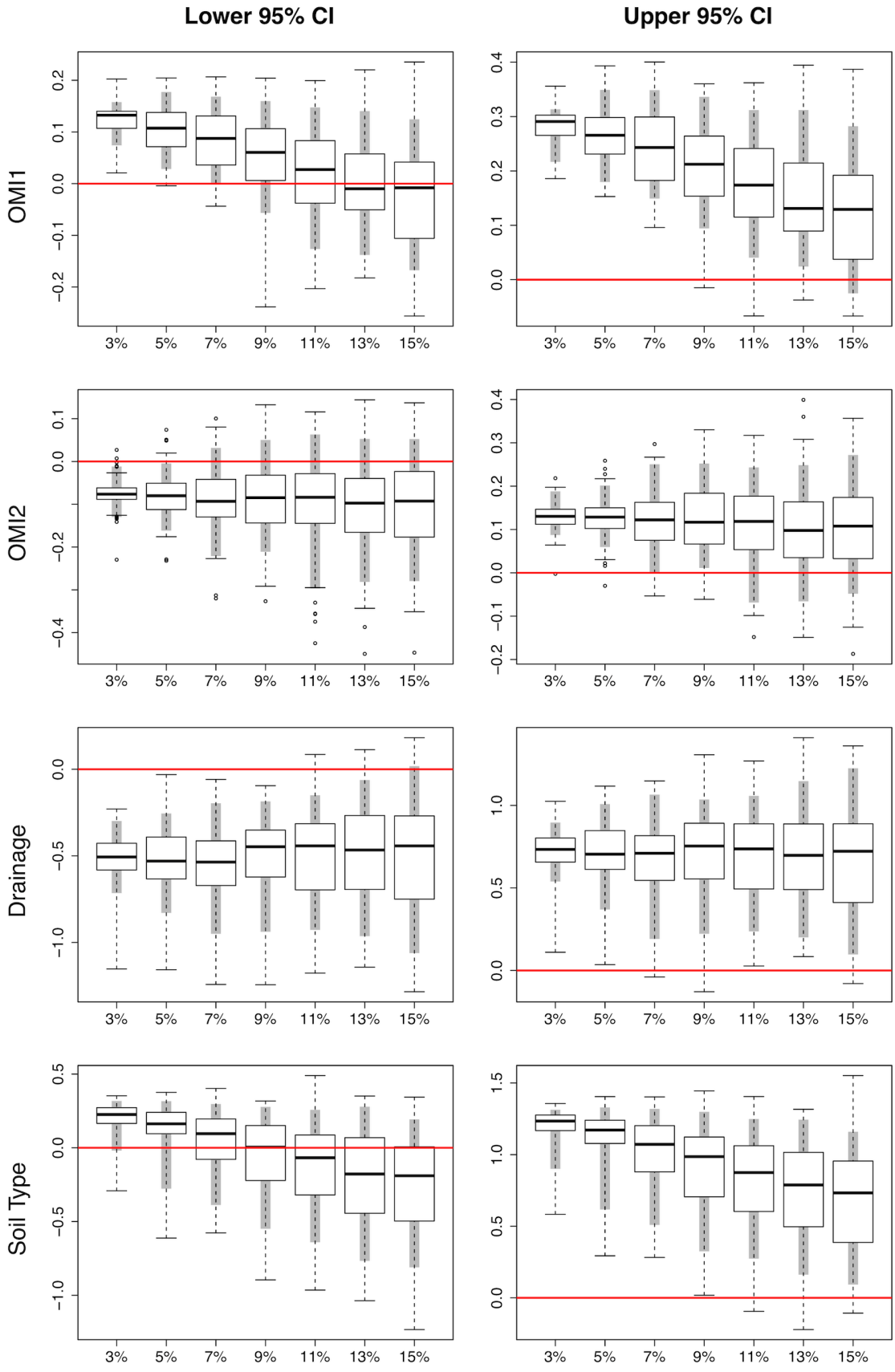

Figure S4 Sensitivity results. Boxplots of the critical estimates for every applied statistical test. The grey bars indicate the range between the 5% and 95% quantiles (i.e. 90% confidence intervals). Results for bedrock type are equal to those of soil type and are not displayed.

CHAPTER III DIFFERENTIAL EFFECT OF FIRE-SURVIVAL STRATEGY ON DIVERSIFICATION AMONG A REPLICATED RADIATION ON TWO CONTINENTS

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Evolution of fire-survival strategies (reseeding and resprouting) has been proposed as a factor explaining the exceptional plant species richness found in Mediterranean areas. Recent research indicates that these strategies could differently affect rates of species diversification. However, little is known about the generality of the effects of differing fires-survival strategies on species evolution.

We study this question in the Restionaceae, a family that radiated in Southern Africa and Australia. The radiations occurred independently and are used as evolutionary replicates. We apply Bayesian approaches to estimate trait-specific diversification rates and climatic niche evolutionary patterns. We also compare the climatic heterogeneity of South Africa and Australia.

Reseeder species diversify faster than resprouters in South Africa, but not in Australia. We further show that climatic niche evolves at a faster rate in reseeders than in resprouter lineages and that the niche optima are different between the two strategies. We find that South Africa was more climatically heterogeneous than Australia independently of the spatial scale considered.

We propose that rapid shifts between fire-survival strategies can promote speciation by separating species ecologically but this likely only happen if the landscape is sufficiently heterogeneous.

Introduction

Fire impacts the current distribution of plant species in several of the world major's biomes (Bond *et al.*, 2005). Many of the current terrestrial hotspots of biodiversity harbour flammable vegetation (Sauquet *et al.*, 2009), and fire is thought to be a key factor explaining the high species richness in these regions (Cowling *et al.*, 1996; Linder, 2003; Barraclough, 2006; Simon *et al.*, 2009). In fire-prone environments, lineages have developed strategies to survive fire by having either parts of the plant surviving, or fire-stimulated germination that allow persistence in regularly burned habitats (He *et al.*, 2011). Adaptive responses to cope with fire can evolve over very short evolutionary timescales (Gómez-González *et al.*, 2011), and fire-survival traits have been shown to be extremely labile on species-level phylogenetic trees (Schnitzler *et al.*, 2011). Therefore, fire can impact plant evolution, but the relation between the appearance of fire-survival strategies and evolutionary success is still controversial. For example, rates of species diversification were not associated with fire-survival strategy in five genera of four families of plants living in fire-prone regions (Verdú *et al.*, 2007). To the contrary, switching between fire-survival strategies can enhance speciation in *Podalyriae* (Schnitzler *et al.*, 2011). Such contrasting results call for additional analyses to complement the patterns found and inform us whether general statements can be made regarding the evolutionary processes involved in fire prone areas.

Adaptations to fire in Mediterranean climate regions can be classified into two main fire response syndromes (Bell, 2001). Resprouter plants survive fire as individuals and then replace the lost structures by growing back from unburnt tissues. In contrast, reseeder individuals die as a result of fire and the population is re-established by a new generation growing from seeds that remained dormant until the occurrence of fire (Bell, 2001). While both strategies are viable, they may differentially affect species evolution. Resprouting is similar to clonal reproduction, whereas reseeders experience a new generation after each fire event as it creates a complete turnover of the populations (Ojeda *et al.*, 2005). The outcome of this direct link between fire events and generation time is that reseeders are expected to have shorter generation times than resprouters (Wells, 1969; Verdú *et al.*, 2007). This important difference in a main life-history trait could have strongly impacted on reseeders and resprouters evolution. Indeed, generation time is negatively correlated with molecular substitution rates in angiosperms (Smith & Donoghue, 2008) which allowed herbaceous species (usually short-lived) to explore a wider climatic space than longer-lived species

because of higher rates of niche evolution (Smith & Beaulieu, 2009). Furthermore, a positive relationship between molecular substitution and diversification rates has been found in birds and reptiles (Eo & Dewoody, 2010), suggesting that species with short generation times may diversify at higher rates than others. Recombination rates could also differ between reseeder and resprouter species because these strategies are comparable to sexual or asexual reproduction, respectively. More frequent recombination in reseeders could thus lead to more rapid genetic change and also increase the rate of trait evolution (Rieseberg *et al.*, 2003; Glémin *et al.*, 2006). Therefore, different fire survival strategies likely play an important role in plant species diversification and comparative study of the resprouter-reseeder evolution should improve our understanding of the effect of life history traits on macroevolutionary processes.

Reseeder and resprouter plants also exhibit numerous ecological differences. A positive relationship has been reported between the presence of resprouters and fire frequency (Bellingham & Sparrow, 1997; Vesk & Westoby, 2004; Lamont *et al.*, 2011; Russell-Smith *et al.*, 2012). Fire frequency is itself linked to climate (Wilson *et al.*, 2010). In a simulation model, only a mild-Mediterranean climate (moderate summer drought and reliable winter rain) favors the replacement of resident resprouters by an invading reseeder (Ojeda *et al.*, 2005). Observations of post-fire succession in South African fynbos vegetation shows that differences in micro-habitat, in this case the presence of seeps, increases the rate of canopy cover for reseeders, while resprouters have an advantage in dryer areas (Rutherford *et al.*, 2011). In many plant lineages, sister-species differ in their fire-survival strategy (Van der Niet & Johnson, 2009; Schnitzler *et al.*, 2011). Thus, sister species likely differ in environmental preferences and these differences facilitate species diversification by ecologically separating populations having different fire-survival strategies.

In this study, we assess the effect of fire-survival strategies on species diversification using Restionaceae (order Poales) as a study system. The Restionaceae are graminoid plants that are ecologically important in oligotrophic heathlands of the southern regions of the African and Australian continents (Linder *et al.*, 2003). These plants radiated independently in both regions with circa 150 species in Australia and New Zealand (subfamilies Sporadanthoideae and Leptocarpoideae; Briggs & Linder, 2009) hereafter referred to as the Australian clade, and 350 species in South African subfamily Restionoideae, which is the third largest clade in the South African Cape flora (Goldblatt & Manning, 2000; Linder, 2003). Reseeders and resprouters are found in an even ratio among Restionaceae and only a handful of species

display both fire-survival strategies (Bell, 2001). As a speciose and ecologically diverse family, the Restionaceae constitute a perfect study system to examine the effect of fire-survival strategies on species evolution, using a densely sampled phylogeny and modern phylogenetic comparative methods. First, we evaluate whether as predicted reseeders diversify at a higher rate than do resprouters. After describing the climatic niche of every species and assessing the climatic heterogeneity found in South Africa and Australia, we test whether reseeders and resprouters are selected towards different climatic optima. Finally, we ask whether reseeders experience a higher rate of climatic niche evolution than do resprouters, which is expected given their shorter generation time and the subsequent expected increased rate of molecular recombination. All analyses are performed on the South African and Australian clades separately to enable a continental comparison of diversification processes.

Materials and Methods

Phylogenetic Inference

We assembled four available plastid gene regions (*atpB*, *matK*, *rbcL* and *trnL-F*) for 382 out of the 496 Restionaceae species and four *Anarthria* species (Anarthriaceae), designated as outgroup taxa following Linder *et al.* (2003), to infer the phylogenetic tree of Restionaceae (Supporting Information Table S1). We broadened this dataset by sequencing a nuclear gene (*phyB*) for 99 species (Supporting Information Table S1; for primers used see Christin et al., 2012) The 50 ml PCR reaction mixture contained ~100 ng of genomic DNA template, 8 ml of GoTaq Reaction Buffer, 3 ml of each dNTP (2.5μM), 1ml of each primer (10μM), 2.5 ml DMSO, and 1 unit of Taq polymerase (GoTaq DNA Polymerase, Promega, Madison, WI). The samples were incubated for 3 min at 94°C, followed by 39 cycles consisting of 40 sec at 94°C, 70 sec of annealing at 57.5°C, and 75 sec at 72°C. The last cycle was followed by a 7 min extension at 72°C. PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN GmbH, Germany) and sequenced using the Big Dye 3.1 Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions, and were separated on an ABI Prism 3100 genetic analyzer (Applied Biosystems). All newly generated sequences have been deposited in the EMBL database (accession numbers indicated in Table S1).

DNA sequences were aligned using MAFFT (Katoh *et al.*, 2002) and ambiguously aligned nucleotides were removed using Gblocks with default settings (Talavera & Castresana, 2007). The single gene alignments were concatenated into a combined matrix of 386 species and

7246 nucleotides. We reconstructed the phylogenetic tree with BEAST (Drummond & Rambaut, 2007), which allows the estimation of substitution rates jointly with divergence times. Absolute dates are not required in this study, but we nevertheless chose to apply an uncorrelated molecular clock using a calibration point (Scholtz, 1985; Linder *et al.*, 2003) on the crown node of the Restionaceae with a lognormal prior (mean=2, stdev=1, offset=64, 5%=64.43, 95%=102.3). The best model of substitution for each partition (Supporting Information Table S2) was identified using MrAIC (Nylander, 2004). Four analyses were run independently in BEAST 1.7.4 for $50*10^6$ generations each. Convergence was verified using Tracer (Drummond & Rambaut, 2007), ensuring that the effective sample size (ESS) of each parameter was always higher than 200 and that the four runs converged adequately. The first 10,000 trees were removed as the burn-in period and a maximum credibility tree was inferred using TreeAnnotator (Drummond & Rambaut, 2007).

Diversification Rate

Of the 383 Restionaceae species present in the present data set, 275 (Australia: 75/146; South Africa: 200/350) had available information concerning their fire-survival strategy (150 reseeders; 125 resprouters). We extracted the fire-survival strategies from the interactive key of Linder (2011) for the South African species and from the literature for the Australian taxa (Meney & Pate, 1999).

We compared the rates of diversification between reseeder and resprouter species of the monophyletic Australian and South African Restionaceae by performing two independent analyses using the BiSSE model, as implemented in the R package diversitree (FitzJohn *et al.*, 2009; R Development Core Team, 2012). This model estimates speciation, extinction, and character transition rates associated with binary traits (Maddison *et al.*, 2007). We corrected for incomplete taxon sampling (FitzJohn *et al.*, 2009) without specifying the fire strategy of the missing species as this information was lacking for both continents. The models were run for 10^6 generations using exponential priors for the rates. We checked for optimal convergence of the runs by verifying the trace files and by using diagnostic metrics available in the R package Coda (Plummer *et al.*, 2010). We calculated the diversification rate by subtracting the extinction rate from the speciation rate. While reseeders and resprouters in Australian Restionaceae show easily identifiable morphological differences (Meney & Pate, 1999), the fire survival strategy of South African Restionaceae is difficult to diagnose in the field. The fire survival strategy data was compiled by one of the authors (HPL) using field observations, herbarium specimens and literature comments (Linder, 2011). To measure what

impact a potential bias in trait determination could have had, we performed a sensitivity analysis (following Wüest et al. submitted). The analysis consisted in running the BiSSE analysis on a set of data obtained by permuting randomly the fire survival strategy of a given percentage of species. The percentage ranged from 2 to 20% in 2% increments and each was replicated 100 times.

Climatic Niche Description and Continental Heterogeneity

We retrieved occurrence records for Southern Africa from several herbaria and for Australia and New Zealand from the Global Biodiversity Information Facility (GBIF) web portal (http://www.gbif.org/). The dataset consists of 23'859 occurrences (12'266 for South Africa and 11'593 for Australia and New Zealand) with, on average, 53 records per species. We used the 19 bioclimatic variables from the Worldclim database at a resolution of 2.5 minutes (Hijmans *et al.*, 2005) to describe the climatic niche of species. Additionally, we created layers for potential evapotranspiration (PET). We derived the PET layers from radiation layers created in GRASS GIS (GRASS development team, 2010) using the empirical equation of Jensen & Haise (1963) We used the "raster" package (Hijmans & Etten, 2012) in R to extract the climatic values for all Restionaceae occurrences and used these data to perform an Outlying Mean Index ordination (OMI; Dolédec et al., 2000) OMI ordination neither assumes a specific shape of species response curves along climatic gradients nor down-weights species with low abundances. Therefore, OMI is well suited for estimating species climatic niches (Dolédec *et al.*, 2000).

We compared climatic heterogeneity in South Africa and Australia by first projecting the climatic layers (both OMI axes and PET) in space. We then used a moving window analysis that took for each focal pixel the standard deviation of a number of adjacent pixels (i.e. within the window). This standard deviation gives a measure of local climatic heterogeneity. The local heterogeneity (at the level of the focal pixel) was then divided by the mean heterogeneity measured in each region to give a measure of the relative spatial heterogeneity available at the spatial scale analyzed. Relative heterogeneity has the advantage of describing the heterogeneity of a pixel independently of the global heterogeneity of a region. It is thus a more robust index in the case where, for example, heterogeneity is spatially aggregated. The fact that many regions of South Africa and Australia are not inhabited by Restionaceae could lead to an estimate of climatic heterogeneity that does not relate to environments in which our focal family radiated. Therefore, we restricted comparison of climatic heterogeneity between continents to pixels that are located in quarter degree squares with known Restionaceae

occurrences. We performed this analysis using multiple window sizes (3 to 103 pixels corresponding to 7.5 arc minutes to ~4.3°) to understand whether local climatic heterogeneity changes with spatial scale.

Evolutionary Processes

We assessed the effect of fire-survival strategy on the climatic niche evolution of the Restionaceae by fitting seven models of character evolution using the OUwie R package (Beaulieu *et al.*, 2012). The first two models were based on Brownian Motion (BM), the first being a single rate BM model (BM1 thereafter) and the second being a BM model with a separate rate σ^2 for each fire-survival strategy (BMS; O'Meara et al., 2006) The five additional models were based on the Ornstein-Uhlenbeck (OU) model, which models the evolution of a character that is pulled with a selective strength α, toward an optimum θ (Hansen, 1997; Butler & King, 2004). While BM models neutral evolution, OU models are used to test for directional evolution in the trait of interest and are especially well suited for studying the evolutionary dynamics of the climatic niche (Salamin *et al.*, 2010) The first OU model assumed a single optimum for all Restionaceae species (OU1). The second OU model (OU_M) estimated separate climatic niche optima for each fire-survival strategy while keeping σ^2 and α constant. The following models optimized for each fire-survival strategy a separate selective optimum and either the corresponding σ^2 rate parameter (OU_{MV}) or selective strength α (OU_{MA}). Finally, the last model optimized for each fire-survival strategy all of the possible parameters of the OU model (OU_{MVA}). All models were applied using the "non-censored" approach (O'Meara *et al.*, 2006) that relies on phylogenetic trees having the evolutionary history of a binary character mapped on the branches (Bollback, 2006). We used the R package Phytools (Revell, 2012) to infer the stochastic mapping of fire-survival strategy evolution on the maximum credibility dated tree from the BEAST analysis. To take into account the uncertainty in reconstructed histories, we replicated the inference 100 times. We analyzed sequentially and independently for the Australian and South African clades each climatic variable (OMI-axis 1, OMI-axis 2 and PET) that describes the main climatic gradients. We recorded the likelihoods for each model and used AIC and Akaike weights to identify the model that best describes our data.

Results

Phylogenetic Inference

The inferred phylogenetic tree (Fig. 1a) is highly similar to previous analyses of the Restionaceae (Hardy *et al.*, 2008) and we found that the topology inferred with phyB was congruent with the cpDNA. As previously found (Johnson & Briggs, 1981; Briggs *et al.*, 2000; Linder *et al.*, 2000, 2003), the tree splits at the root of the family in two monophyletic clades containing Australian and South African species, respectively. The estimated age of the root of the Restionaceae in the present study (~80my) is at the upper end of the range of estimates published elsewhere (Wikström *et al.*, 2001; Bremer, 2002; Janssen & Bremer, 2004). The phylogeny is generally highly supported with the majority of nodes having posterior probabilities higher than 0.95 (Fig. 1b,c).

Diversification Rate

The MCMC chains from independent runs adequately converged, and the effective sample sizes of the estimated parameters are high (>10.000 for every parameter), which indicates only little autocorrelation between the samples. In the South African clade, the 95% credible intervals of the posterior distribution of speciation rates do not overlap, confirming that reseeders have a significantly higher speciation rate than resprouters (Fig. 2a). On the other hand, overlapping credible intervals for speciation rates in the Australian clade indicate similar rates of speciation in resprouters and reseeders (Fig. 2b). Extinction rates do not differ between fire survival strategy in both continents (Fig. 2c,d). We find that reseeders diversify four times faster than resprouters in South Africa (Fig. 2e). The net diversification rate for resprouters is close to zero, which is included in the 95% credible interval of the posterior distribution. Diversification rates do not differ significantly between reseeders and resprouters in the Australian clade (Fig. 2f). Moreover, there is no diversification rate difference between Australian species and South African resprouters. The inferred transition rates between both fire strategies and the extinction rates are similar between the two continents (Fig. 2g,h).

The sensitivity analysis (Supporting Information Fig. S1, Fig. S2) shows that the results are robust towards a potential sampling bias in the data. Indeed, in the South African clade, the speciation rate of reseeders is still significantly higher than that of resprouters even if 5% of the species have been swapped to the other group. The speciation rate difference disappears only when 15% or more of the species are reassigned to the other strategy. In the Australian

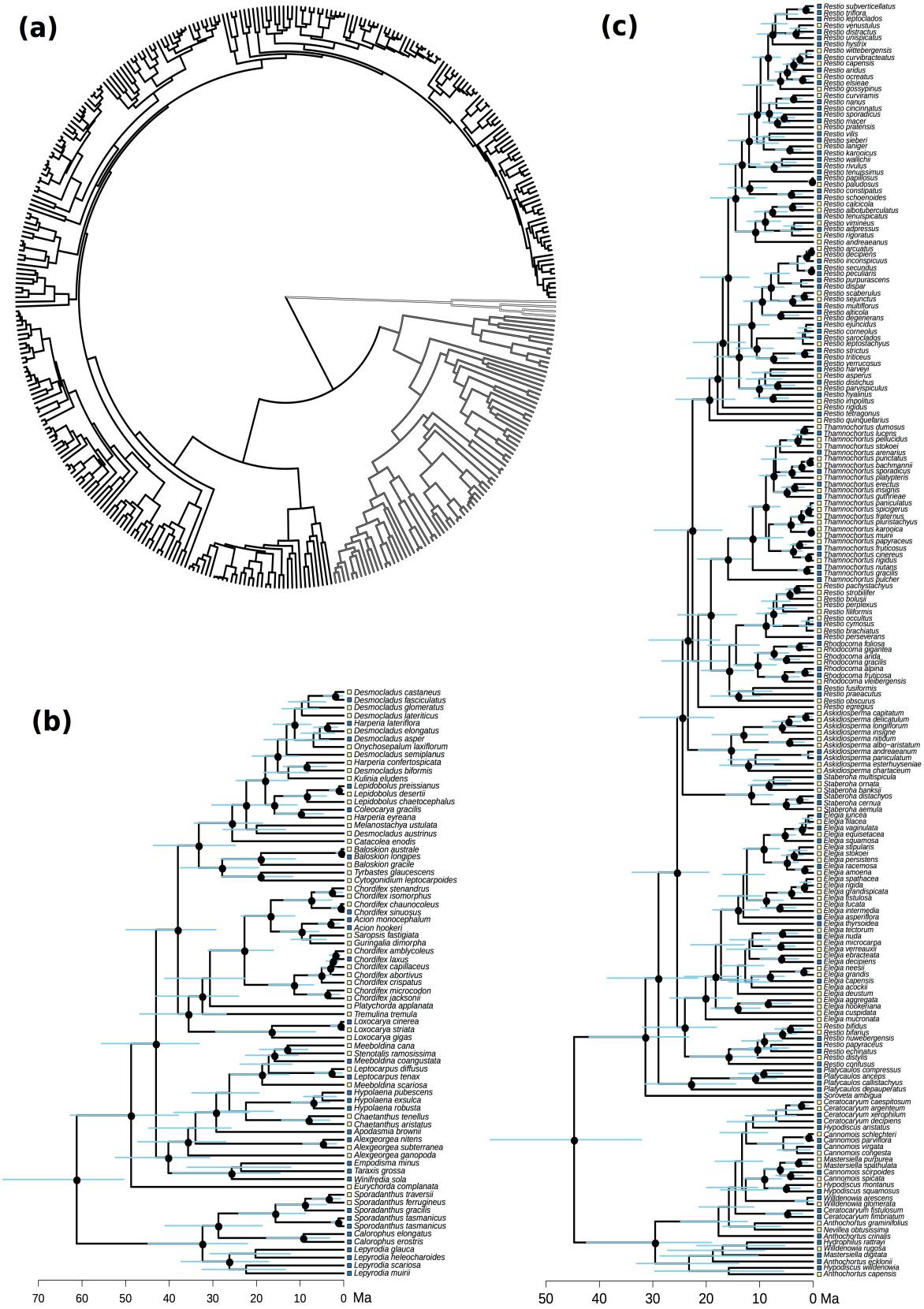

Figure 1 Maximum clade credibility tree of the Restionaceae (a). The branches belonging to the outgroups are colored in white. The Australian clade is highlighted in grey in panel a and shown in detail in panel b. The South African clade is shown in black in panel a and in detail in panel c. In panels b and c, credible intervals for the age estimates are shown and nodes with posterior probabilities above 0.95 are identified by dots. Colored boxes next to species names refer to the fire-survival strategies (yellow, reseeder; blue, resprouter).

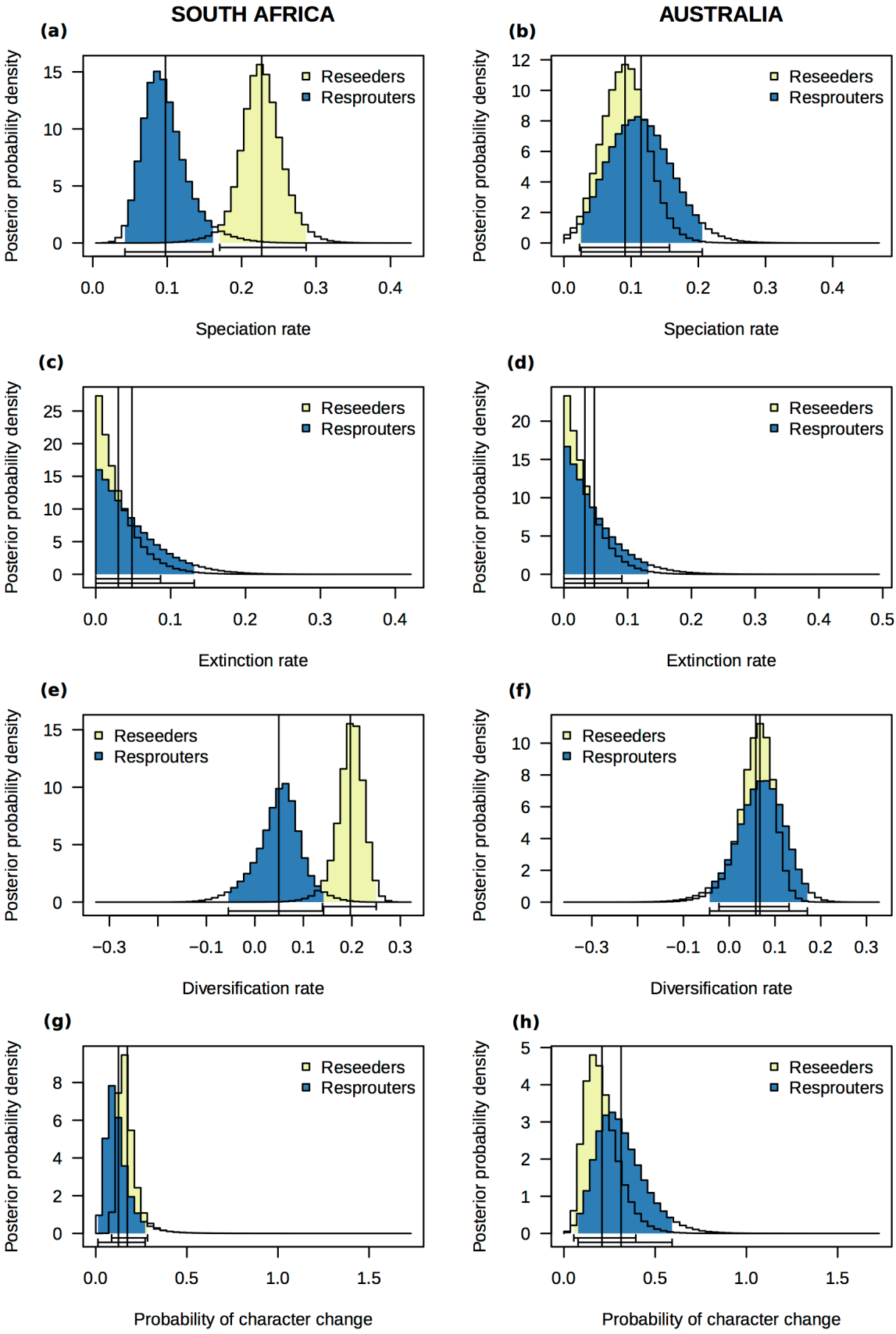

Figure 2 Posterior probability densities of the inferred parameters from the BiSSE analyses performed on the Australian (panels a, c, e & g) and South African clade (panels b, d, f & h), separately. Diversification rate (panels e & f) is calculated by subtracting extinction (panels c & d) from speciation rates (panels a & b). Reseeders are shown in yellow and resprouters in blue.

clade, the reseeders and resprouters never differ in their diversification rates, suggesting that it is unlikely that a random assignment of the fire-strategy trait could create the pattern we found in South-Africa.

Climatic Niche and Evolutionary Processes

The first two axes of the OMI ordination explain 45% and 27 % of the total variance, respectively, and were used in subsequent analyses. The first axis (OMI1) is mainly driven by precipitation variables, with low values indicating wet conditions with low seasonality (temperature and precipitation) and high values indicating highly seasonal low-precipitation conditions. The second axis (OMI2) displays a temperature gradient ranging from hot at the lower end to cold conditions at the upper end. We find that South Africa is two times more heterogeneous for OMI1 and OMI2 and three times for PET than Australia (Fig. 3). Changing the spatial scale considered does not affect the results (Fig. 3).

We present the analyses on evolutionary models using Akaike weights to show the relative support for each model (Table 1). In the Australian clade, the best models describing the evolution of climatic preferences are BM1 for OMI1, OU_M for OMI2 and OU_{MA} for PET. In South Africa, OU_{MV} is the best model for OMI1 and PET while, as in the Australian clade, OU_M has the highest support for OMI2 (Table 1). The picture given by the OU models with multiple optima shows reseeders being selected towards colder and less seasonal climates with higher PET than resprouters (Table 2, Table 3). The pattern is consistent between the two continents. Out of the seven models, three also infer the rate of evolution (σ^2) of the characters. In all cases except one (PET in Australia), reseeders have a higher relative rate of evolution than resprouters (Table 2, Table 3).

Discussion

Fire-survival strategy is a trait that has often been used to explain the high species richness found in the Cape Floristic Region (Linder, 2003; Barraclough, 2006; Schnitzler *et al.*, 2011). Shifts in fire-strategies between closely related species have the potential to simultaneously contribute to a reduction in gene flow while allowing for divergent selection (Servedio *et al.*, 2011). Ecological divergence may emerge because of differences in the environmental conditions associated with different fire regimes (Ojeda *et al.*, 2005). Our results reveal a strong link between fire-survival strategies and diversification patterns in the Restionaceae, but only in the South African clade (Fig. 2). We show that reseeders and resprouters occupy

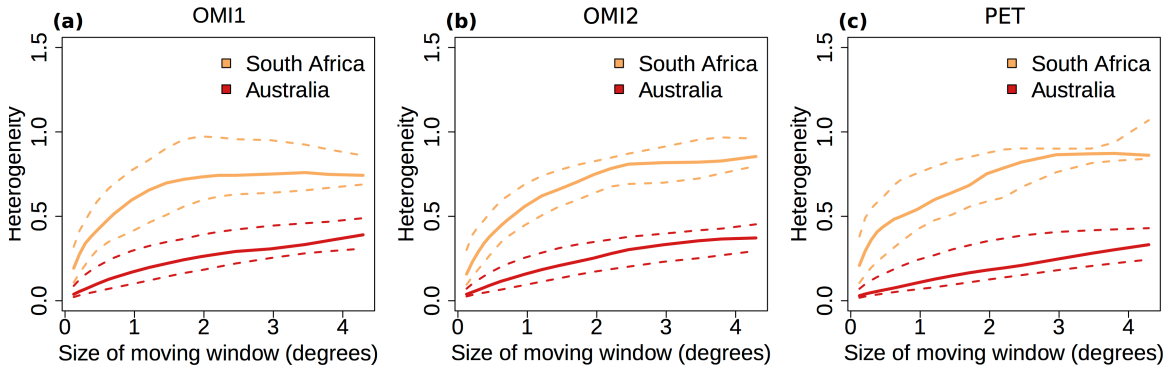

Figure 3 The spatial heterogeneity of the the first (a) and second (b) OMI axis and PET (c). Mean heterogeneity is shown with a solid line and dashed lines represent the first and third quantiles. Heterogeneity of Southern Africa is shown in black and Australia is shown in grey. Spatial scale is shown on the x axis which gives in degree the size of the moving window used in the analysis.

Table 1 Comparison of the fit of two Brownian motion models (BM) and five Orstein-Uhlenbeck models (OU) of climatic niche evolution.

		South Africa				**Australia**			
	Model	lnL	AIC	ΔAIC	ωi	lnL	AIC	ΔAIC	ωi
OMI	BM1	-281.92	567.91	83.09	0	**-150.13**	**304.45**	**0**	**0.36**
axis	BMS	-281.31	568.74	83.92	0	-149.42	305.19	0.75	0.25
1	OU1	-240.71	489.62	4.8	0.04	-148.96	306.52	2.07	0.13
	OU_M	-240.45	489.11	4.29	0.05	-149.41	307.43	2.98	0.08
	OU_{MA}	-238.47	487.24	2.42	0.12	-148.34	307.6	3.15	0.07
	OU_{MV}	**-237.26**	**484.82**	**0**	**0.41**	-148.26	307.43	2.99	0.08
	OU_{MVA}	-236.29	485.01	0.19	0.38	-147.91	309.13	4.69	0.03
OMI	BM1	-317.37	638.81	45.64	0	-141.18	286.54	40.44	0
axis	BMS	-316.59	639.3	46.13	0	-140.13	286.62	40.52	0
2	OU1	-293.13	594.46	1.29	0.16	-118.81	246.22	0.12	0.29
	OU_M	**-292.48**	**593.17**	**0**	**0.3**	**-118.75**	**246.1**	**0**	**0.31**
	OU_{MA}	-291.81	593.93	0.76	0.2	-118.14	247.2	1.1	0.18
	OU_{MV}	-291.57	593.44	0.27	0.26	-118.27	247.46	1.36	0.15
	OU_{MVA}	-291.63	595.69	2.52	0.08	-117.8	248.91	2.8	0.08
PET	BM1	-116.32	236.7	58.94	0	-92.22	188.62	18.38	0
	BMS	-115.71	237.55	59.8	0	-89.67	185.7	15.45	0
	OU1	-85.93	180.07	2.31	0.11	-82.05	172.72	2.47	0.1
	OU_M	-85.7	179.61	1.86	0.14	-81.65	171.91	1.67	0.14
	OU_{MA}	-84.61	179.53	1.77	0.14	-80.01	170.94	0.7	0.23
	OU_{MV}	**-83.72**	**177.76**	**0**	**0.35**	-80.19	171.31	1.07	0.19
	OU_{MVA}	-82.97	178.39	0.63	0.26	**-78.46**	**170.24**	**0**	**0.33**

different climatic optima along both OMI axes and PET in South Africa (Table 2), while in Australia this is only the case along the second OMI axis and PET gradient (Table 1, Table 3). Moreover, we show that South-African climate is more heterogeneous, regardless of the spatial scale we consider (Fig. 3). The different selective optima linked with the fire survival strategies most likely drive diversification through ecological speciation (Schluter, 2000; Rundle & Nosil, 2005) in the Cape Floristic Region. In contrast, we could not observe the same trend in the evolution of the Australian Restionaceae. The discrepancy among continents in the effect of fire-survival strategy on diversification shows the importance of the ecological context (e.g. climatic heterogeneity) in which species are diversifying.

Table 3 Estimates of the rate of climatic niche evolution (σ2), strength of selection (α) and selective optimum (θ) with credible interval of the Australian dataset for all models and stochastic mappings replicates. Models with the higher support (ωi; see Table 1) are shown in bold.

	Model	Sigma reseeder	Sigma resprouter	Alpha reseeder	Alpha resprouter	Theta reseeder	Theta resprouter
OMI	**BM1**	**0.235±0.04**					
axis	BMS	0.376±0.308	0.086±0.329				
1	OU1	0.296±0.078		0.012±0.011			
	OU_M	0.295±0.079		0.013±0.011		-1.375±1.283	-1.402±1.372
	OU_{MA}	0.2±0.101		0.014±0.012	0.005±0.014	-1.452±1.209	-1.31±1.285
	OU_{MV}	0.452±0.444	0.09±0.48	0.011±0.012		-1.424±1.358	-1.334±1.393
	OU_{MVA}	0.026±0.354	0.312±0.604	0.015±0.017	0.011±0.017	-1.382±1.286	-1.352±1.321
OMI	BM1	0.183±0.031					
axis	BMS	0.32±0.148	0.004±0.15				
2	OU1	1.053±0.77		0.305±0.229			
	OU_M	**0.979±0.741**		**0.282±0.221**		**-1.16±0.274**	**-1.178±0.209**
	OU_{MA}	0.019±0.083		0.206±0.135	0.178±0.121	-1.282±0.225	-1.068±0.222
	OU_{MV}	1.035±1.043	0.541±1.1	0.269±0.207		-1.18±0.256	-1.187±0.214
	OU_{MVA}	0.035±0.141	0.036±0.693	0.2±0.112	0.18±0.109	-1.209±0.224	-1.167±0.213
PET	BM1	0.046±0.008					
	BMS	0.078±0.045	0±0.053				
	OU1	0.093±0.027		0.06±0.02			
	OU_M	0.096±0.032		0.064±0.026		5.852±0.234	5.842±0.227
	OU_{MA}	0.027±0.035		0.07±0.029	0.052±0.039	5.831±0.19	5.835±0.183
	OU_{MV}	0.135±0.107	0±0.122	0.057±0.025		5.796±0.237	5.863±0.23
	OU_{MVA}	**0±0.002**	**0.876±2.14**	**0.102±0.023**	**0.056±0.027**	**5.757±0.275**	**5.865±0.23**

Table 2 Estimates of the rate of climatic niche evolution (σ2), strength of selection (α) and selective optimum (θ) with credible interval of the South African dataset for all models and stochastic mappings replicates. Models with the higher support (ωi; see Table 1) are shown in bold.

	Model	σ^2 reseeder	σ^2 resprouter	α reseeder	α resprouter	θ reseeder	θ resprouter
OMI	BM1	0.116±0.012					
axis	BMS	0.124±0.015	0.094±0.02				
1	OU1	0.305±0.072		0.205±0.052			
	OU_M	0.304±0.07		0.205±0.051		0.115±0.147	0.171±0.097
	OU_{MA}	0.282±0.054		0.184±0.035	0.176±0.034	0.089±0.147	0.155±0.114
	OU_{MV}	**0.382±0.093**	**0.188±0.056**	**0.209±0.048**		**0.112±0.115**	**0.165±0.102**
	OU_{MVA}	0.395±0.136	0.076±0.076	0.195±0.048	0.208±0.046	0.086±0.106	0.129±0.103
OMI	BM1	0.166±0.017					
axis	BMS	0.182±0.022	0.124±0.032				
2	OU1	0.355±0.065		0.127±0.027			
	OU_M	**0.355±0.065**		**0.129±0.027**		**0.948±0.158**	**0.904±0.259**
	OU_{MA}	0.349±0.063		0.122±0.025	0.117±0.025	0.934±0.225	0.889±0.276
	OU_{MV}	0.395±0.082	0.27±0.08	0.128±0.027		0.945±0.166	0.9±0.236
	OU_{MVA}	0.39±0.099	0.29±0.246	0.129±0.028	0.127±0.027	0.941±0.173	0.857±0.222
PET	BM1	0.022±0.002					
	BMS	0.024±0.003	0.018±0.004				
	OU1	0.049±0.009		0.146±0.032			
	OU_M	0.049±0.01		0.147±0.032		5.508±0.085	5.485±0.051
	OU_{MA}	0.055±0.012		0.164±0.037	0.158±0.037	5.495±0.06	5.492±0.051
	OU_{MV}	0.059±0.013	0.035±0.009	0.152±0.033		5.494±0.07	5.489±0.053
	OU_{MVA}	**0.067±0.021**	**0.014±0.012**	**0.139±0.033**	**0.152±0.031**	**5.493±0.059**	**5.483±0.058**

Fire-Survival Strategies and Diversification Rates

The hypothesis that reseeders have a higher speciation rate than resprouters has been proposed some time ago (Wells, 1969). We tested this hypothesis by comparing the difference in rates of speciation of reseeders versus resprouters using the BiSSE model (Maddison *et al.*, 2007). We found that these reproductive strategies differ significantly in speciation rates in the South African clade, where the diversification rate was higher for reseeders than resprouters compared to their Australian counterparts (Fig. 2e,f). The other parameters of the model (rate of extinction and character change) do not differ between fire-survival strategies and continents (Fig. 2c,d,g,h). The posterior distribution of resprouter diversification rates (whether South African or Australian) included zero (Fig. 2e,f). This could be interpreted as diversification mostly occurring in reseeder lineages. Schnitzler et al. (2011) found no

difference in rate of speciation between fire-survival strategies in *Protea* (Proteaceae) and the legume tribe Podalyriae. Statistical power issues due to a smaller number of species in phylogenies, as well as unknown clade-specific factors, may explain why no speciation rate difference was found between reseeders and resprouters in these two plant groups. Nevertheless, fire-survival strategy is the most important factor for Podalyriae speciation (Schnitzler *et al.*, 2011), showing that even if diversification rates do not differ between strategies, fire is still a main factor promoting speciation in the Mediterranean climate regions.

Climatic Niche Evolution

We inferred the patterns of climatic niche evolution by fitting models of increasing complexity starting from simple BM to OU models with different optima, selective strength and rates of evolution between fire-survival strategies. We showed that reseeder Restionaceae are selected towards colder and less seasonal climates than resprouters in South Africa. This result corroborates well studies made on different plant lineages that include the same fire survival strategy syndromes (Ojeda *et al.*, 2005; Russell-Smith *et al.*, 2012). The phylogenetic framework that we report in this study allows us to model distinct optima for each fire survival strategy, and to model the Brownian rate of evolution underlying the selective process (Beaulieu *et al.*, 2012). We found that when the evolutionary models allow distinct rates of evolution between resprouters and reseeders, the latter displays faster rates of evolution (except for the variable PET in Australia; Table 2, Table 3). These results parallel the general finding that herbaceous plants, similar to the reseeders, have short generation times and experience higher rates of climatic niche evolution than woody taxa, which as resprouters show longer generation times (Smith & Beaulieu, 2009). While reseeders of South Africa and Australia all share higher rates of climatic niche evolution than do resprouters, we find a higher rate of speciation only in South African reseeders.

Context Dependent Ecological Speciation

In this study we show the variable effect of fire-survival strategies on Restionaceae diversification using two replicate radiations on two continents. Reseeders generally show a higher rate of climatic evolution than do resprouters, which allows them to evolve rapidly into different habitats. Thus, the great ecological diversity in the South African Restionaceae is most likely the result of ecological diversification taking place in reseeder species (see next paragraph for discussion on the even ratio of extant reseeders/resprouters). We find no difference in speciation rate or selective optima along OMI axes, representing climatic

gradients, between the two fire-survival strategies in Australia. Linder et al. (2003) propose several explanations to elucidate the different radiation pattern between South African and Australian Restionaceae. While these authors ruled out morphological differences and variable climatic histories that would explain the discrepancy between continents, they propose that higher speciation rates in South Africa are possibly best explained by the steeper altitudinal and climatic gradients found in the Cape Floristic Region compared to the Australian landscape (Cowling *et al.*, 1996; Linder *et al.*, 2003). Here, we compare climatic heterogeneity between South Africa and Australia and find that independently of the spatial scale, climatic heterogeneity is always higher in South-Africa than in Australia (Fig. 3). We propose that the climatic heterogeneity in Australia is not sufficient to allow for efficient ecological speciation in the Australian Restionaceae. We also find that only South African reseeder species have a higher rate of speciation, while South African resprouters share similar rates with Australian Restionaceae. Assuming that extinction rates are well estimated (Rabosky, 2010), our results rule out the possibility that Australian Restionaceae have lower extinction rates, as Linder et al. (2003) suggested. The paucity of selection towards different climatic optima in Australia could explain why reseeders do not show accelerated rates of diversification there.

Even though reseeder taxa exhibit a higher rate of diversification in South Africa, a similar number of extant reseeder and resprouter species has evolved in both continents. This can be explained by high rates of transition between fire-survival strategies during evolution. Indeed, both strategies can replace one another in local communities, depending on climate and fire regimes (Ojeda *et al.*, 2005), and the morphological adaptation to a different fire regime can take place quickly (Gómez-González *et al.*, 2011). The transition rates between the two strategies are similarly elevated on the two continents and are also nearly equal in both directions (Fig. 2g,h), which explains the similar numbers of reseeder and resprouter species. Indeed, when new species appear, a shift from one strategy to the other can easily occur. The greater number of reseeders obtained through species diversification is thus counter-balanced because a greater number of those will change into resprouters than the reverse (because of their initial larger number). This will result in an equal proportion of reseeder and resprouter that stays constant through time.

The framework we used in the study allows us to better understand why the link between fire-survival strategies and diversification rate is not universal among plant groups (e.g. Verdú et al., 2007). Indeed we show that the ecological context (here climatic heterogeneity) is a key

component that will likely impact on plant diversification patterns. The resprouting strategy is well known to be one of persistence allowing to survive disturbance events (Bond & Midgley, 2001). As resprouters species survive in unforeseeable environments, reseeders are more likely to specialise to particular ecological conditions. This fragile equilibrium may be disrupted under global change (Westerling *et al.*, 2006; Wilson *et al.*, 2010). In this case, resprouters may be privileged over reseeders in the short term because they are more likely to persist in unpredictable climates than reseeders (Clarke *et al.*, 2010), which could lead to a radical change in the evolutionary trajectory of the radiation.

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Supporting Information

Table S1 This supplementary table contains accession numbers of the sequences of the dataset. The table is not included in the thesis, but is available from the author upon request.

Table S2 Gene-specific models selected under AICc.

Data set	AICc model
atpB	K2P+Γ
matK	GTR+Γ
phyB	GTR+I+Γ
rbcL	K2P+I+Γ
trnL-F	HKY+Γ

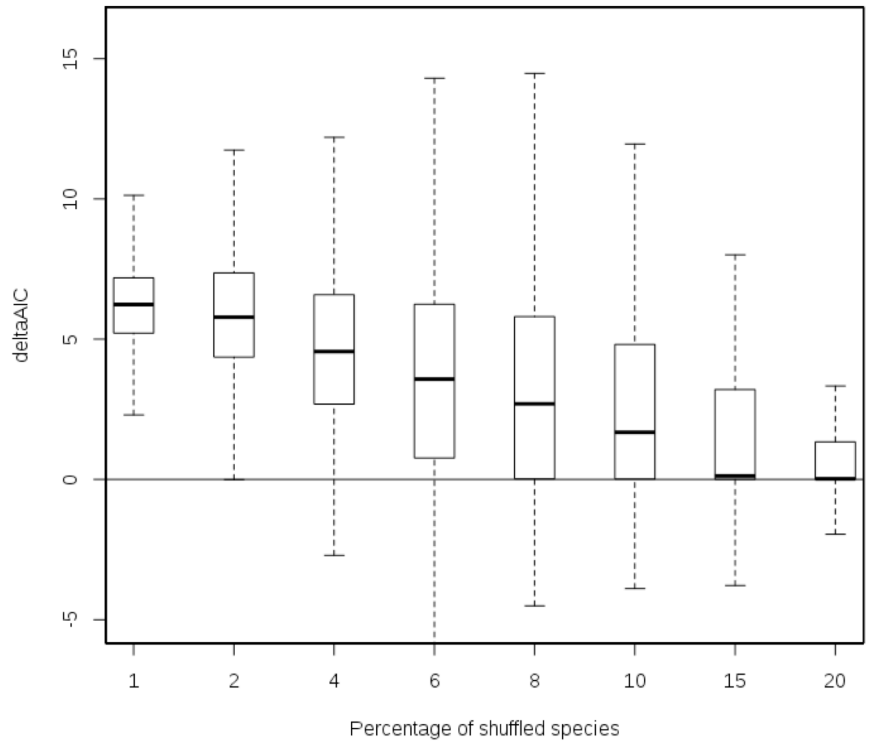

Figure S1 Sensitivity analysis of the BiSSe analysis done on the South African clade. Difference in AIC values (y-axis) between the model inferred with the real data and replicates done with a certain number (x-axis) of species fire survival strategy randomly switched to the opposite state. Each boxplot represents the results of 1000 replicates.

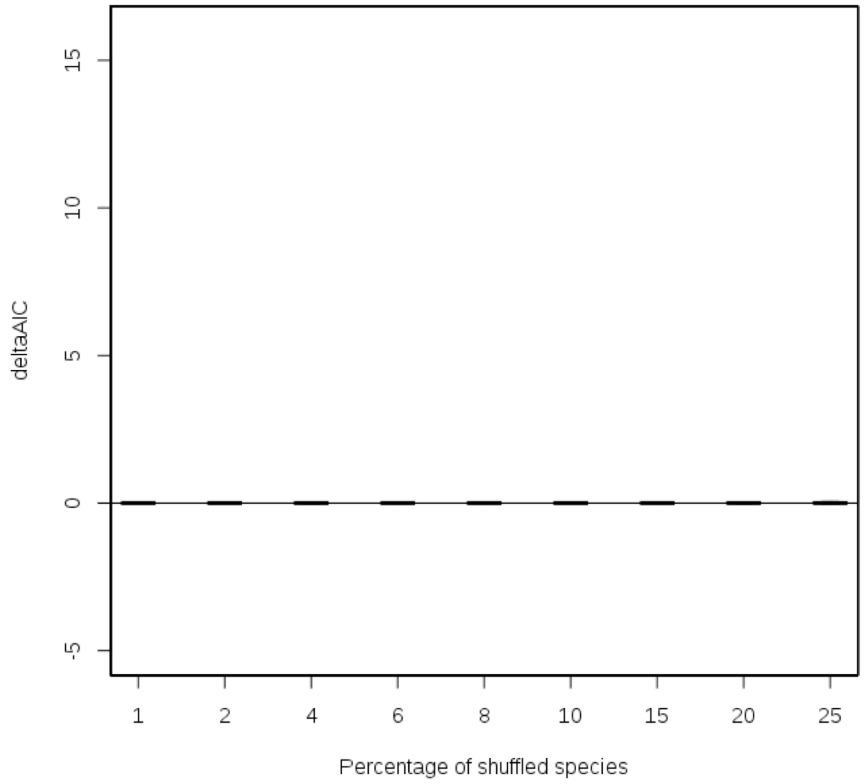

Figure S2 Sensitivity analysis of the BiSSe analysis done on the Australian clade. Difference in AIC values (y-axis) between the model inferred with the real data and replicates done with a certain number (x-axis) of species fire survival strategy randomly switched to the opposite state. Each boxplot represents the results of 1000 replicates.

CHAPTER IV GRIME REVISITED: ASSEMBLY OF SOUTH AFRICAN RESTIONACEAE ALONG AN ENVIRONMENTAL GRADIENT

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We use an integrative ecophylogenetics framework to test whether the importance of competition in structuring local Restionaceae (Poales) assemblages decreases with increasingly harsh environmental conditions in the Cape Floristic Region. The framework integrates statistically-optimized sampling and linear mixed models to assess simultaneously the effects of environmental gradients, species pool definition, and phylogenetic scale on the estimated phylogenetic structure of local assemblages. We show that phylogenetic clustering decreases with increasing drought stress, consistent with competition playing a minor importance in assemblages in harsh environments. Analysis under the framework reveals that phylogenetic scale affects estimated phylogenetic structure of local assemblages more than does species pool composition. Random effects of species pools indicate a shift in the relative importance of local versus regional assembly processes at a spatial scale of ca. 50x50km. The low variance explained by mixed models suggests that, in addition to environment-dependent strength of competition, effects of fire history or soil characteristics could impact assemblages of Cape Restionaceae. The integrative ecophylogenetics framework provides a flexible, feasible approach for studying the interaction between biotic and abiotic factors, local and regional processes, as well as taxon-specific effects in regulating species assembly in a range of organisms and ecosystems.

Introduction

Identification of the mechanisms that create and maintain species diversity in and among local species assemblages forms the principle challenge in community ecology. Niche theory suggests that the occurrence of species depends on both abiotic conditions and biotic interactions (Hutchinson 1957, Chase and Leibold 2003). Likely, current abiotic conditions, ongoing biotic interactions, and historical processes that influence the composition of regional species pools, interact in complex ways in determining the composition of local species assemblages (Ricklefs 1987). Species vary in their preferences for and tolerances of abiotic conditions, which act as filters to exclude species from some assemblages (e.g. Kearney and Porter 2004, Chown and Gaston 2008) (but see Hubbel 2001). Biotic interactions, competition and facilitation in particular for terrestrial plants, can also drive biodiversity patterns within and among local assemblages (Schoener 1983, Connell 1983). However, the question of how the importance of biotic interactions in structuring species assemblages, varies with changing abiotic conditions is still open.

Preferences and tolerances of species along environmental gradients can influence how species interact and alter the importance of those interactions for community structure. For example, under distinct temperature regimes competition between two stream salmonid fishes leads to different dominant species (Taniguchi and Nakano 2000). In plants, environmentally determined change from facilitation to competition between exotic and native prairie grasses alters native species richness and cover (Richardson et al. 2012). Also, experimentally altered soil silicon content leads to a shift in the competitive balance between the two grass species Poa annua and Lolium perenne, mediated by herbivory (Garbuzov et al. 2011). In an attempt to formalize ideas regarding changing biotic interactions along environmental gradients, Grime (1973, 1979) suggested that competition among plants has decreasing importance in shaping species assemblages along a gradient of increasing environmental harshness (herafter reffered to as Grime's hypothesis). In contrast to favorable environments, harsh environments are ones in which disturbance is high or productivity is low due to stress induced by factors such as drought or nutrient deficiency (Grime 1973, 1979 p. 20). Experiments, as well as field studies support this hypothesis (e.g. Wilson and Keddy 1986, Campbell and Grime 1992, Brose and Tielbörger 2005). Coupled distribution models of Fagus sylvatica also suggest that competition decreases under less favorable environmental conditions (Meier et al. 2011). Despite these supportive results, the generality of Grime's hypothesis has been questioned on grounds of theory (Tilman 1982, Chesson and Huntly 1997), methodology (Grace 1993) and

meta-analysis (Goldberg et al. 1999). We revisit the debate on Grime's hypothesis by making use of the rapid development in the field of ecophylogenetics over the last decade (reviewed in Cavender-Bares et al. 2009, Mouquet et al. 2012).

The field of ecophylogenetics integrates phylogenetic information with community ecology. Ecophylogenetic studies quantify the phylogenetic structure of local communities, which reflects the relatedness of coexisting species and is expected to vary with evolutionary patterns in traits (conserved vs. labile) and the dominant assembly process (environmental filtering vs. competitive exclusion; Webb et al. 2002). For example, under the assumption of high phylogenetic signal, environmental filtering should result in coexisting species that are closely related (phylogenetic clustering; e.g. Webb 2000, Swenson et al. 2006, Cavender-Bares et al. 2006). Similarly, the contrasting outcome of distantly related co-occurring species is expected when competitive exclusion is the dominant assembly process (phylogenetic overdispersion; e.g. Slingsby and Verboom 2006, Helmus et al. 2007). Thus, in the ecophylogenetic framework, two ingredients are required to predict the phylogenetic structure of communities: (i) information on the evolution of traits that are relevant in mediating the assembly of local species assemblages, and (ii) information on the importance of alternative assembly mechanisms (environmental filtering vs. competitive exclusion). While information on trait evolution is becoming available for numerous species due to the rapid development of phylogenetics and comparative biology, obtaining strong evidence for community assembly mechanisms is expensive and time consuming, ideally involving manipulative experimental approaches that can only address small numbers of species. As an alternative approach that can be applied to biologically diverse communities and assemblages, we apply Grime's hypothesis to predict how phylogenetic structure of local assemblages changes along environmental gradients.

A prediction how phylogenetic structure of local assemblages changes along environmental harshness gradients will depend on the evolutionary pattern in the assembly-relevant traits. For now, we assume conserved evolution in the adaptation of our study clade towards tolerating harsh environmental conditions. This leads us to expect phylogenetic clustering in harsh environments because the relevant traits, adaptations to persist in harsh environments, are phylogenetically conserved and the dominant assembly process in harsh environments is environmental filtering according to Grime's hypothesis (compare with Table 1 in Webb et al. 2002 p. 479). Following Grime's rationale, moving away from the harsh end of an environmental gradient will lead to an increasing relative importance of competition in

assembling species (Grime 1973, 1979). This increasing importance of competition leads to a successive shift from clustering to overdispersion along the gradient, because phylogenetic overdispersion is expected if competition is dominant assembly process (cf. Webb et al. 2002). Therefore, we expect a positive slope when plotting phylogenetic clustering against environmental harshness (solid line Figure 1). In case the assembly-relevant traits are not phylogenetically conserved but rather exhibit a highly labile, convergent pattern of evolution, we should expect the inverse pattern along the harshness gradient because communities are expected to be clustered in favorable conditions and random in harsh conditions (dashed line Figure 1; Webb et al. 2002). This illustrates how one can use simple hypothesis to predict how phylogenetic structure of local assemblages changes with environment using the ecophylogenetics framework.

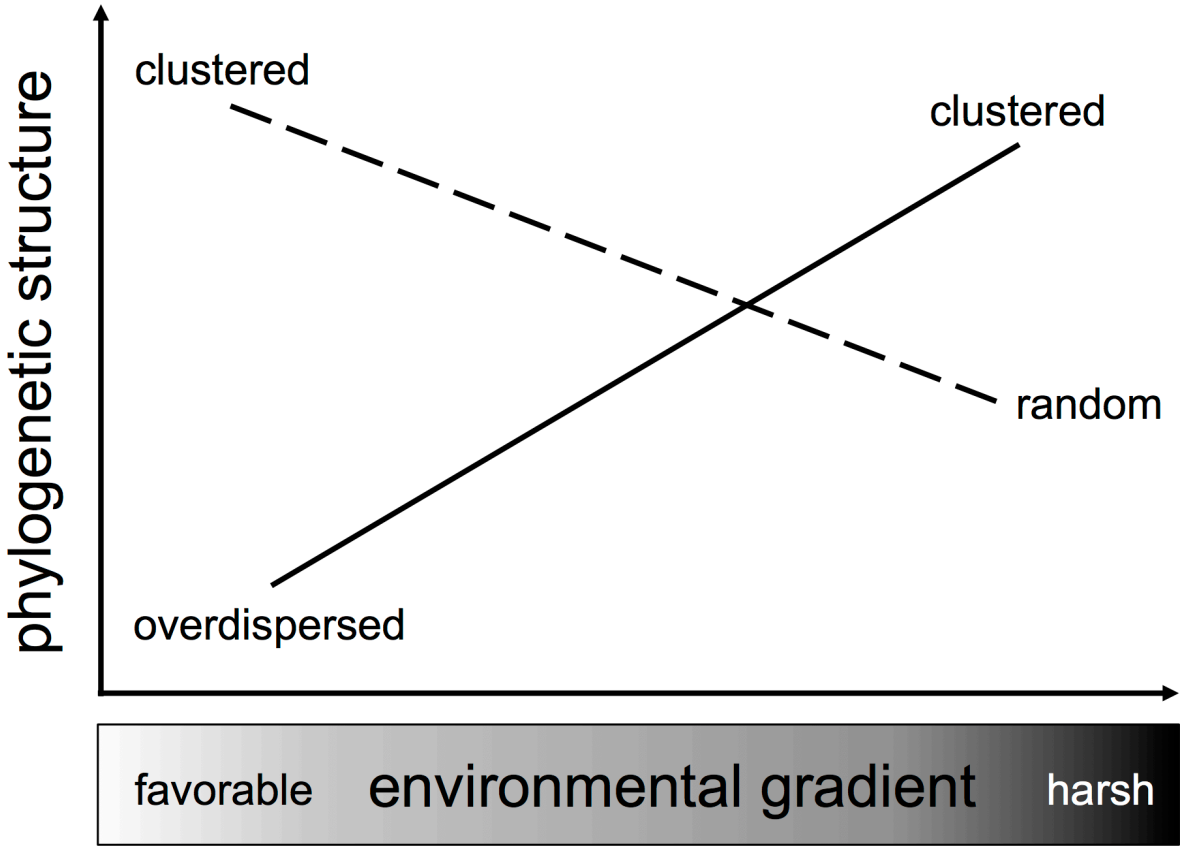

Figure 1 Predicted scenarios of how phylogenetic structure of local assemblages varies along environmental harshness according to Grime's hypothesis in the case of phylogenetically conserved (solid line) and convergent evolution (dashed line) of traits.

The rapidly increasing ecophylogenetics literature over the last decade identifies several potential pitfalls (Cavender-Bares et al. 2009, Mouquet et al. 2012). First, ecophylogenetic analysis relies on the assumption that local assemblages of species are a subsample of a regional source pool. Studies based on both simulated (Kraft et al. 2007) and observational data (e.g. Swenson et al. 2006, Cavender-Bares et al. 2006, Lessard et al. 2012) demonstrate that the probability of detecting phylogenetic clustering increases with source pool size. Second, phylogenetic scale influences the outcome of ecophylogenetic studies such that phylogenetic overdispersion is more likely at finer taxonomic scales (Cavender-Bares et al. 2006, Vamosi et al. 2009). Source pool size and phylogenetic scale are, with a few exceptions, regarded as inherent properties of a study rather than explanatory variables in ecophylogenetic analysis. We suggest to integrate both the phylogenetic scale and source pool size as explanatory variables, vary them following a full factorial design, and use mixed effect models to statistically assess their effects. Linear mixed effect models take into account the non-independence that arises when repeating ecophylogenetic analyses at multiple phylogenetic scales and source pool sizes. While meta-analysis leads us to expect that phylogenetic clustering increases with phylogenetic scale (Vamosi et al. 2009), inference on the direction and magnitude of the source pool size effect is mixed (reviewed in Vamosi et al. 2009, see also Lessard et al. 2012, González-Caro et al. 2012). Our approach offers the possibility to integrate the two neglected factors into ecophylogenetics.

In this study, we investigate whether the assembly of local Restionaceae (Poales) assemblages of the South African Cape Floristic Region (CFR) is consistent with Grime's hypothesis. The South African Restionaceae are part of the Restionaceae family that consists of ca. 450 species worldwide (Linder et al. 1998) and are ideally suited to investigate community assembly processes along environmental gradients for multiple reasons. First, an updated species level phylogeny (Litsios et al. submitted) that is based on previous phylogenetic assessments of the Restionaceae (Eldenas and Linder 2000, Moline and Linder 2005, Hardy et al. 2008) confirmed the South African Restionaceae constitute a monophyletic clade that evolved 60-70 Ma (Linder et al. 2003). Second, the clade diversified into ca. 350 species (Linder 2003) that exhibit great ecological diversity. Third, the clade occurs throughout the CFR, providing an ideal situation in which the species are distributed across the large environmental gradients of the CFR. Finally, continuous updates on the taxonomy of the Cape Restionaceae (Linder 1984, 1985, Linder and Hardy 2010) lead to an interactive identification key (Linder 2011) that allows identification of the morphologically diverse group. We use information on trait evolution and Grime's hypothesis to predict how the phylogenetic

structure of Cape Restionaceae assemblages changes along an environmental harshness gradient. The linear mixed effect model approach allows us to assess and compare how environmental harshness, assembly processes, source pool size, and phylogenetic scale affect estimates of the phylogenetic structure of local assemblages. Assessing the importance of assembly mechanisms over large environmental gradients allow us to better understand the origin and maintenance of spatial structure in biodiversity; a demanding task, especially in an exceptionally diverse region such as the CFR.

Materials and Methods

Study System

The study area included the Greater Cape Floristic Region in South Africa (Born et al. 2006). We used data on assemblages of species in the Restionaceae which were collected following a hierarchical, stratified random sampling scheme (Wüest et al. in review). The sampling scheme ensured that the local assemblage data (i) spanned the major climatic gradients of the CFR and (ii) covers small scale variation in habitats. The sampling was carried out in austral spring and summer years 2009-2011 and yielded 235 local assemblages with a mean species richness of 3.7 Restionaceae species per plot and 127 species in total.

Environmental Harshness Gradient

We used moisture balance (MBAL), a measure that integrates potential evapotranspiration (PET) and precipitation (PREC), as an index of environmental harshness for plants. PET reflects the potential evaporative demand defined by incoming energy (radiation and temperature) and, therefore, increases with increasing energy. PET on its own does not reflect environmental harshness for plants because its effect on plants varies with precipitation. While high PET conditions in combination with low precipitation could cause drought stress in plants when evaporative demand is greater than water supply, the same PET conditions would not be problematic for the same plants in combination with high precipitation. We calculated MBAL as ratio of PET to PREC so that low MBAL values (low PET and high PREC) corresponded to favorable, and high MBAL values (high PET and low PREC) to harsh environmental conditions, respectively.

We calculated average monthly PET at a resolution of 30 arc seconds using the empirical equation of Jensen and Haise (Jensen and Haise 1963), which we derived from monthly potential solar radiation layers created in GRASS GIS (GRASS Development Team 2010)

and monthly mean temperature from the WorldClim database (Hijmans et al. 2005). From these monthly PET layers, we estimated annual average MBAL for all sampling locations throughout the CFR.

Species Pools

We assembled eight potential species pools for each quarter degree square (QDS, 0.25° by 0.25°) in order to assess the effect of species pools on our ecophylogenetic analyses. The **sample** species pool refers only to the total of species sampled in the specific assemblage (sample plot). This produces the smallest possible species pool. On the other extreme, the **global** species pool encompasses all South African Restionaceae of the CFR. The remaining species pools are of intermediate size between the two extremes and refer to an entire QDS. The **observations** species pool consists of all the species that were ever recorded in the QDS, including the collected herbaria occurrences and our sampling. The **biogeographic** species pool is based on the phytogeographic centers of the CFR (Goldblatt and Manning 2000). For this pool, every species that was recorded in one phytogeographic zone was assigned to each QDS that was fully or partially covered by that zone. Furthermore, we analogously built three species pools based on a comprehensive map of South African vegetation (Mucina and Rutherford 2006). We followed the nested hierarchy comprised of vegetation types (**vegtype** 165 classes) within **bioregions** (33 classes) within **biomes** (at the lowest resolution, 4 classes in total).

In addition to the map-based approaches, we also estimated the **models** species pool using ensemble species distribution modeling. We modeled the distribution of species for which we had >10 occurrences using four different modeling algorithms (GLM, GAM, BRT, and MaxEnt) and 8 different parameterizations in R (R Development Core Team 2012). The parameterizations consisted of 8 different combinations of the bioclimatic variables, PET, and radiation (for more details see Appendix A). Since most of the modeling techniques require data on species absences, we randomly generated 15000 pseudo-absence points for each species within the CFR. We chose this number because it sufficiently represented the variability in the study region along major climatic gradients (Appendix B). We assigned species to the species pool of a QDS if at least 75% of the models (24 out of 32) predicted presence in at least one pixel within the QDS, using the TSS maximized threshold (Allouche et al. 2006).

Ecophylogenetics

We assessed the phylogenetic pattern in MBAL preferences using a occurrence database that consisted of approximately 12,000 geo-referenced records from the herbaria BOL and NBG, assembled by Linder. We extracted MBAL conditions for all occurrences and estimated preferred MBAL conditions for each species as the median along the MBAL gradient. Divergent evolution of PET preferences in accordance with highly labile reseeder/resprouter life history trait in Restionaceae (Litsios et al. submitted) could indicate the same pattern of phylogenetically convergent evolution in MBAL preferences. If so, we could expect MBAL preference in Restionaceae to exhibit low phylogenetic signal, which we tested using Pagel's λ (Pagel 1999), because it performed well in a comparison of phylogenetic signal measures (Münkemüller et al. 2012). However, low phylogenetic signal alone is not proof of convergent evolution. We used phylogenetic generalized linear models in the caper R package (Orme et al. 2012) to take into account phylogenetic relationships among species when estimating whether reseeders and resprouters differ in MBAL preference using the. In Restionaceae, the reseeding and resprouting strategies are phylogenetically highly labile. Thus, a significant difference in MBAL preferences, taking into account the phylogenetic relationship among species, would indicate convergent evolution of adaptations to drought stress.

We used a set of previously inferred, dated phylogenies (Litsios et al. submitted) to study the phylogenetic structure of Restionaceae assemblages. We repeated all analyses on a set of 100 trees in order to account for phylogenetic uncertainty. The 100 trees constituted a random sample from the posterior distribution of trees derived from a Bayesian divergence time analysis that was performed on four chloroplast and one nuclear gene regions in the BEAST software (Drummond and Rambaut 2007). The phylogeny encompasses 301 out of the ca. 350 Restionaceae species present in the Cape Floristic Region and spans all genera. We pruned the phylogeny and the community data to the set of species present in both, which resulted in 225 communities encompassing a total of 110 species. We used the pruned datasets and phylogeny to calculate net relatedness index (NRI) and nearest taxon index (NTI) as measures of phylogenetic structure in local species assemblages with the help of the picante package (Kembel et al. 2010) in R. NRI and NTI quantify phylogenetic structure by comparing the phylogenetic relatedness of species within a community against a null expectation and, thus, represent standardized measures of the phylogenetic structure in a given species assemblage (Webb et al. 2002). NRI measures the mean pairwise phylogenetic distance of all species in a

given assemblage standardized by random expectation, whereas NTI is based on phylogenetic distances to the nearest taxon in the phylogenetic tree (Webb et al. 2002). Both measures indicate phylogenetic clustering (species are more closely related than expected) as they increase above zero and overdispersion (more distantly related species than expected) as they decrease below zero. We repeated NRI and NTI calculation for the entire South African Restionaceae clade (hereafter referred to as Restionaceae) and for the Restioneae clade only (a monophyletic clade across all trees that constitute a subfamily; Briggs and Linder 2009), in order to assess how phylogenetic scale affects the estimation of phylogenetic structure in local assemblages.

Phylogenetic signal in species frequency, i.e. number of times species occur in the community dataset, can lead to decreased power and increased type I errors in detecting phylogenetic structure of local assemblages (Kraft et al. 2007, Hardy 2008, Kembel 2009). We therefore tested for phylogenetic signal in the abundance of species at both phylogenetic scales using Pagel's λ (Pagel 1999). We tested for significance of phylogenetic signal in species abundances by comparing with a likelihood ratio test the model of estimated λ with a model with λ enforced to be zero.

The standardization of NRI and NTI relies on a null expectation of phylogenetic structure. This null expectation can be derived from various null models, and selecting the appropriate null model is important in community ecology in general (Gotelli 2000) and in ecophylogenetics in particular because the choice of null model can influence results (Hardy 2008, Kembel 2009). The only null model that allows testing for different species pools is one that randomly draws species from the species pool. Simulation studies showed that this null model produces robust results as long as there is no phylogenetic signal in the frequency of species (Hardy 2008). Our null model randomly drew species from the species pool of the QDS of a given local assemblage to calculate NRI and NTI, thus, allowed incorporation of species pools of different sizes at the QDS level.

Statistical Analyses

We used linear mixed effect models as implemented in the R package lme4 (Bates and Maechler 2011) to investigate Grime's hypothesis and simultaneously incorporate the effect of species pools, phylogenetic scale, and tree uncertainty. Linear mixed effect models are appropriate because they can account for non-independence in variables with random effects. We included NRI values of the Restionaceae assemblages (or NTI values, respectively) as the

response variable and MBAL as a fixed effect. The random effects consisted of QDS identity, source pool type, phylogenetic scale, and tree uncertainty. We included QDS identity as a random effect because data points (phylogenetic structure of a local assemblage) within a QDS share the same species pool and, therefore, are not independent. Measurements of phylogenetic structure for each species pool, for both phylogenetic scales, and for each of the 100 trees reflect repeated measures and are thus best treated as random effects. We introduced all random effects as random intercepts in both models.

There is no consensus how to best calculate a goodness of fit measure like R2 for linear mixed effect models. We, therefore, calculated R2 of the correlation between observed and predicted values (R2CORR; Byrnes et al. 2006) as a measure of how well fitted linear mixed effect models explained variation in the data. There is no straight forward way to test the significance of fixed effects in the linear mixed effect framework because of difficulties in accurately estimating the denominator degrees of freedom for random effects (Bolker et al. 2009). Ergo, we assessed the significance of the fixed effects by inspecting the 95% highest posterior density intervals from a Markov Chain Monte Carlo sample (n=1000) of the model coefficients. Assessing the significance of random effects using classical likelihood ratio tests could result in conservative estimated p-values because the tested parameter is at the boundary of parameter space (Bolker et al. 2009). Therefore, we followed an alternative approach implemented in the R package RLRsim (Scheipl et al. 2008) to estimate the significance of random effects. We inspected normal QQ plots for normality of the residuals.

Results

Species Pools

Species richness and spatial variability of species pools differed considerably between species pool definitions (Figure 2, Appendix C). Excluding the **sample** (3.2±2; mean species richness ± standard deviation) and **global** approaches (349 species), median species richness for the species pool definitions ranged from 6 for an **observations** pool to 348 for a **biomes** pool. Both the **bioregions** (277±107) and the **biomes** species pools (326±78) exhibited high values of SR throughout the CFR, resulting in low spatial variability. The **biogeographic** approach resulted in intermediate SR (173±108). The **models** (47±50) and **vegtype** species pools (81±65) came closest in representing the spatial variability in the **observations** pool (18±31, Figure 2).

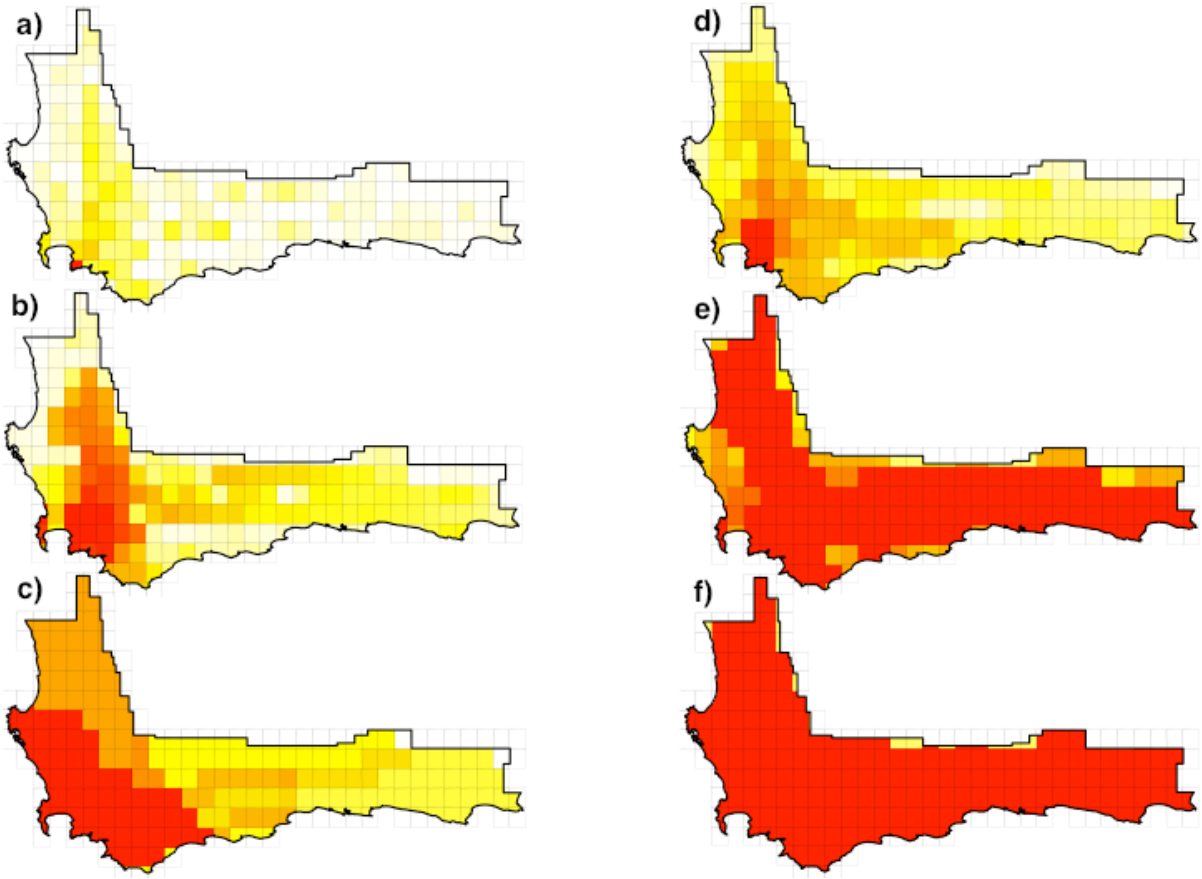

Figure 2 Spatial variation of species richness within and between species pool definitions. The left panel shows the **observations** approach in a), the **models** approach in b), **biogeographic** in c). The right panel shows the results from using vegetation classifications at different resolutions ranging from **vegtype** in d) to **bioregions** in e) to **biomes** in f). The colors represent species richness of the species pool, ranging from white=0 to red=350. The **sample** and **global** species pools are not shown.

Ecophylogenetics

MBAL exhibited low phylogenetic signal (<0.42) that were significantly different from one, consistent over phylogenetic scales (Table 1). Phylogenetic generalized linear models inferred significantly different MBAL preferences for resprouter and reseeders for the majority (75%) of phylogenetic trees in the Cape Restionaceae (Table 1). All phylogenetic tress exhibited significant differences between reseeders and resprouters in MBAL preferences when analyzed in the Restioneae only. The significant differences in MBAL preferences indicate convergent evolution of adaptations to drought stress.

Species frequencies analyzed across the entire CFR Restionaceae revealed low and non-significant phylogenetic signal (Pagel's $\lambda<0.001$, Table 1). Phylogenetic signal slightly increased ($\lambda\leq0.023$) but remained non-significant when inferred for the Restioneae. This

indicates that the results from community phylogenetic analyses are not biased by phylogenetic signal in species abundances and can be considered robust.

Table 1 Phylogenetic signal of species frequencies in the sampled Restionaceae assemblages and of MBAL, repeated for the two phylogenetic scales and a set of 100 trees (random sample). The estimates are reported as median with the range in parentheses, p values are reported as median with the percentage of tree replicates for which p was below the significance threshold (0.05) in parentheses.

	entire Restionaceae		Restioneae only	
	Estimate	p	Estimate	p
Phylogenetic signal (λ)				
Species frequencies[1]	<0.001	1 (0%)	(<0.001) 0.007 (0.023)	0.928 (0%)
MBAL[2]	(0.258) 0.329 (0.413)	<0.001 (100%)	(0.226) 0.273 (0.343)	<0.001 (100%)
Convergence test (PGLS)				
Difference[3]	(0.296) 0.317 (0.349)	0.047 (75%)	(0.356) 0.375 (0.406)	0.032 (100%)

[1] significance assessed from Likelihood Ratio Test, testing whether λ is different from zero
[2] significance assessed from Likelihood Ratio Test, testing whether λ is different from one
[3] difference in MBAL preference between reseeders and resprouter

Measures of phylogenetic structure in the Restionaceae assemblages we sampled ranged from -2.59 to 2.84 (NRI) and -2.47 to 2.97 (NTI) with a mean of -0.09±1.05 (NRI) and -0.03±1.07 (NTI) when analyzed on the entire Restionaceae. When we restricted analysis to the Restioneae, values of NRI and NTI exhibited grand means, averaged over species pools, of 0.30±0.96 (NRI) and 0.24±0.97 (NTI), whereby values ranged from -2.36 to 3.93 (NRI) and -2.72 to 3.33 (NTI).

Statistical Analyses

We observed negative and significant slope estimates of both NRI and NTI along MBAL (Figure 3) as indicated by the confidence intervals that exclude zero (Table 2). All random effects (QDS identity, species pool identity, phylogenetic scale, and tree uncertainty) were significant in both models ($p<0.001$). However, variance components varied markedly in magnitude (Table 2). QDS identity and phylogenetic uncertainty exhibited very high and very low component estimates for both NRI and NTI. The variance component of phylogenetic scale was higher in the NRI compared to the NTI model, whereas we found the opposite for species pool identity: inferred variance component was greater in NTI. The estimated random effect intercepts for each species pool and phylogenetic scale are displayed in Appendix D. The linear mixed effect model explained 21.6% (NRI) and 20.5% (NTI) of the variation in the data, as assessed by R2CORR (Table 2). The residuals were normally distributed.

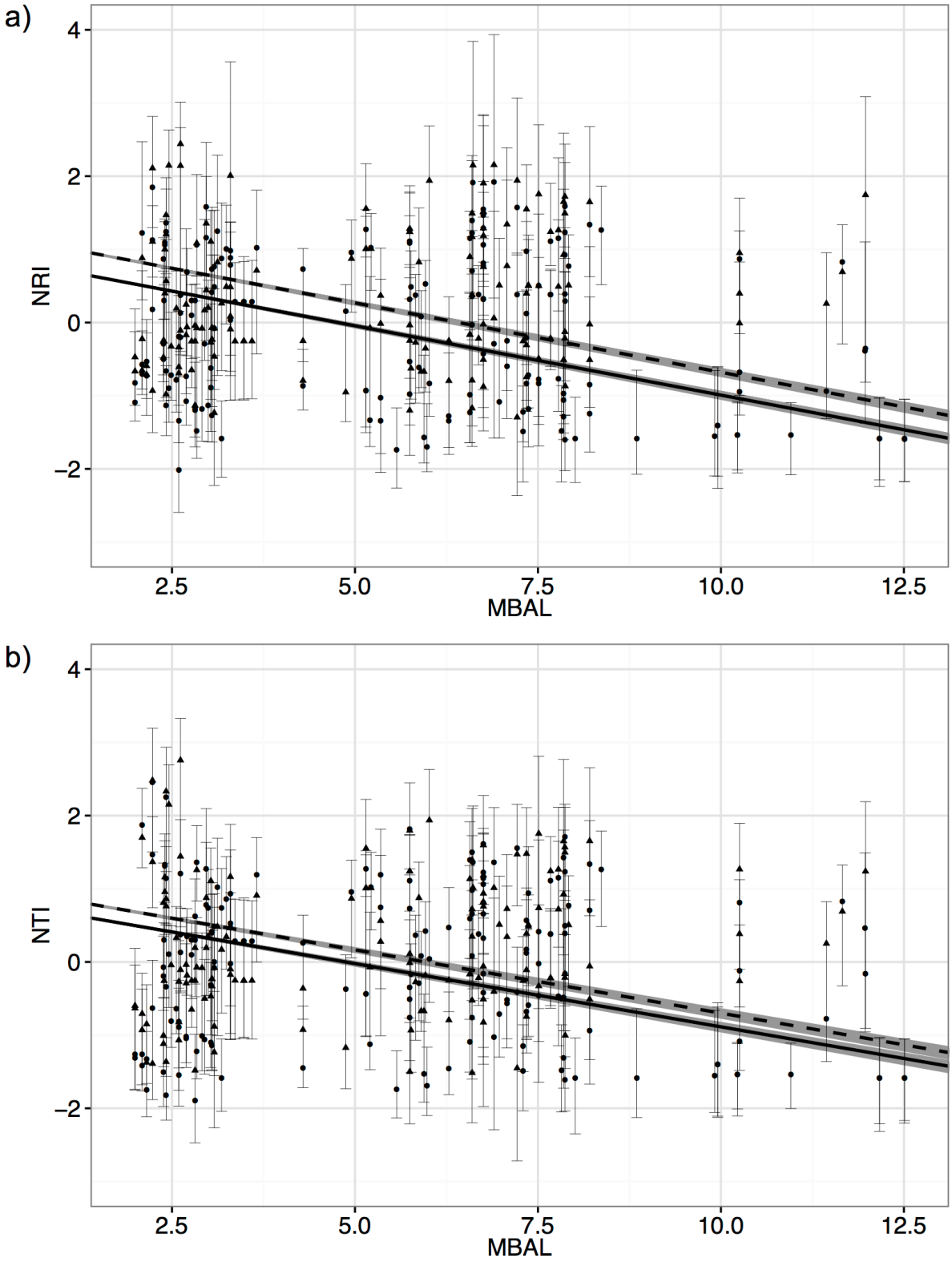

Figure 3 Phylogenetic structure of local Restionaceae assemblages along MBAL. Mean NRI in a) and NTI in b) per site are displayed as circles when based on entire Restionaceae, and as triangles when based on Restioneae only. The total variation in NRI/NTI per site, stemming from phylogenetic uncertainty and pool definition, is indicated by bars. The black lines represent estimated slopes of NRI/NTI along MBAL for the full Restionaceae clade (solid) and the Restioneae (dashed). The grey area indicates uncertainty around the slope estimates, as defined by the posterior confidence intervals.

Table 2 Variance components and fixed effects as estimated by the linear mixed effect models for NRI and NTI. Values of the MBAL estimates in parentheses represent the 95% highest density intervals of the posterior MCMC sample. R^2_{CORR} depicts explained variance.

	NRI	NTI
Variance components (random effects)		
QDS	0.583	0.772
Species pool	0.008	0.014
Phylogenetic scale	0.048	0.045
Tree uncertainty	<0.001	<0.001
Residual	0.830	0.849
MBAL estimates (fixed effect)		
Intercept	(0.513) 1.060 (1.600)	(0.511) 0.937 (1.425)
Slope	(-0.193) -0.190 (-0.186)	(-0.176) -0.173 (-0.170)
R^2_{CORR}	0.216	0.205

Discussion

We outlined how information on (i) the evolutionary pattern in a trait and (ii) the environment-specific importance of alternative assembly mechanisms allows predicting the phylogenetic structure of communities along environmental gradients. In South African Restionaceae, adaptations to drought stress that lead to species specific preferences along the MBAL gradient exhibit a labile, convergent pattern of evolution (Table 1). Thus, we can predict decreasing phylogenetic clustering along MBAL (dashed line Figure 1), given the importance of competition in determining species assembly decreases with environmental harshness (Grime 1973, 1979). Our results are consistent with this prediction because the estimated slopes of NRI and NTI along MBAL are negative (Figure 3). We used an integrative ecophylogenetics framework that incorporates and assesses effects of source pool size as well as phylogenetic scale and uncertainty to estimate NRI and NTI along PET. This approach revealed that phylogenetic scale affected estimated phylogenetic structure to a greater extent than did species pool definition (Table 2), while phylogenetic uncertainty only had a marginal effect. In the following paragraphs, we discuss the consistency of our results with Grime's hypothesis in Restionaceae assembly as well as advantages and implications of the integrative ecophylogenetics framework.

Our results are consistent with the prediction that phylogenetic clustering decreases with environmental harshness, as we find a significant negative slope of both NRI and NTI along MBAL (Table 2, Figure 3). Recent studies that investigate phylogenetic community structure along elevational gradients corroborate our findings (but see Bryant et al. 2008). In both hummingbird and ant assemblages environmental filtering acts on phylogenetically conserved adaptations to harsh high elevation environments to increase phylogenetic clustering with elevation (Machac et al. 2011, González-Caro et al. 2012). Consistent with Grime's hypothesis, these studies conclude that the importance of competition increases towards the more favorable conditions at lower elevations (even though environmental filtering can still be an important assembly process in more favourable conditions; Graham et al. 2012). Our study adds to the growing number of ecophylogenetic studies along environmental gradients that are consistent with Grime's idea that competition is of decreasing importance as a mechanism of community assembly as environmental conditions become harsher.

Explained variance (R2CORR) of the models that estimate NRI and NTI along MBAL is rather low (0.205-0.216). This is not surprising because various environmental factors in addition to MBAL and competition likely influence the assembly of local Restionaceae assemblages. For example, Wüest et al (in review) showed soil type is associated with the ratio of the richness of resprouting versus reseeding Restionaceae species, suggesting a likely influence of soil type on the structure of Restionaceae assemblages. Additionally, varying fire frequencies throughout the CFR affect composition of local assemblages (e.g. Thuiller et al. 2007, Silvertown et al. 2012) and likely contribute to variability around estimated indices of phylogenetic structure. Furthermore, rodent communities in the central Mojave Desert became more phylogenetic clustered with increasing environmental heterogeneity (Stevens et al. 2012). An influence of environmental heterogeneity on the phylogenetic structure of Restionaceae assemblages is likely in the highly heterogeneous Cape Region (Litsios et al. submitted). Small scale differences in soil hydrology potentially also contribute to the observed scatter around the predicted assemblage structure, because hydrological niche segregation influences Restionaceae community structure (Araya et al. 2011). We suggest further research (i) includes these potential sources of unexplained variation in the phylogenetic structure of Restionaceae communities along MBAL and (ii) applies the integrative ecophylogenetics framework to other ecosystems and organisms in order to evaluate the generality of support for Grime's hypothesis.

Ecological realism in the applied species pool definitions varies and each implies the importance of particular ecological processes. For example, the **biogeographic** pool definition implies that dispersal limitation is important in shaping the species pool. In contrast, the modeled relationships between species distributions and climatic conditions constitute the only factor determining species assignment to species pools in the **models** species pools. These differences in the implicit ecological characteristics among species pools can be used to interpret ecophylogenetic analyses. For example, Swenson et al. (Swenson et al. 2006) found an abrupt increase in phylogenetic clustering with increasing spatial scale and suggested that such scaling shifts could identify the scale at which regional processes, such as biogeography or evolution, start dominating local processes in assembling communities (Ricklefs 1987). Also, phylogenetic clustering in North American ant communities decreased when species pools were increasingly constrained in size (Lessard et al. 2012). In our study, estimated phylogenetic clustering based on the **sample** and **observations** species pools appeared to be lowest, as indicated by the large negative random effect estimates in the case of NTI (Appendix D). This could suggest a shift in the relative importance of regional vs. local process in community assembly at the QDS level, because the two species pool definitions are based only on information from within a QDS, while all other species pool definitions use species occurrence information from throughout the cape. However, the **observations** species pool is based on herbarium records and likely underestimates the number of species per QDS, which could partly explain the large negative random effect.

Inferred variance components of species pool identity is greater in the NTI than in NRI model (Table 2). Topological peculiarities of the Restionaceae phylogeny at the larger phylogenetic scale likely explain why species pool's variance component are reduced in the NRI model. The two monophyletic subclades Restioneae and Willdenowieae are separated by very long branches. These long branches bias measures of NRI towards larger values as soon as species from both subclades are present in the species pool because NRI standardization is based on mean phylogenetic distances between all species pairs of a sampled community (Webb et al. 2002). The standardization of NTI, on the other hand, is based on phylogenetic distances between closest relatives only and, therefore, is more sensitive towards subtle changes in the species pool composition than NRI. With regard to species pools, our results endorse that careful selection of the metric used in ecophylogenetics is crucial (Kembel 2009) and highlight the added value of an ecophylogenetics framework that incorporates species pool definitions.

Phylogenetic scale significantly affects ecophylogenetic analyses of local Restionaceae assemblages (Table 2). The fact that the variance component and the estimated random effects of phylogenetic scale are smaller in NTI than in NRI models is consistent with the properties of the two measures. When changing the phylogenetic scale, a measure based on phylogenetic distances of closest relatives (NTI) is less affected than one based on mean pairwise phylogenetic distances of all sampled species (NRI). The random effect estimates indicate that phylogenetic clustering increased when we restricted phylogenetic scale to the Restioneae (Figure 3, Appendix D). This is contrary to results reported on schoenoid sedge communities in the CFR (Slingsby and Verboom 2006). Further, a meta analyses of 39 ecophylogenetic studies (Vamosi et al. 2009) finds that phylogenetic clustering is positively correlated with phylogenetic scale. This discrepancy could be explained by the fact that the majority of ecophylogenetic studies report conserved evolution in assembly relevant traits, contrary to MBAL preferences in Restionaceae. Further research that extends the limited phylogenetic scale replication and expands to other traits and organisms is needed to assess the generality of our results regarding traits that exhibit convergent phylogenetic patterns.

In conclusion, we show that the phylogenetic structure of Restionaceae assemblages along the MBAL gradient support Grime's hypothesis (Grime 1973, 1979) of the decreasing importance of competition to community assembly with increasingly harsh environmental conditions. Further research is needed to elucidate environmental determinants of the observed variation around predicted phylogenetic structure along MBAL in Restionaceae assemblages. An analogous study in the clade that arose from a parallel Restionaceae radiation in Australia appears promising. The integrative ecophylogenetics framework allows detecting shifts in the relative importance of local versus regional assembly processes, above which regional, biogeographic, or evolutionary processes predominantly determine species assembly (Ricklefs 1987). In Restionaceae, this shift manifests at the spatial scale of QDS (ca. 50x50km). In addition, the integrative framework allows assessing the effect of phylogenetic uncertainty, various species pool definitions and multiple phylogenetic scales, which reveals that phylogenetic scale has a greater effect on estimated phylogenetic structure along MBAL than species pool identity. Our study is possibly the first that explores the relative roles of species pools and phylogenetic scale on estimated phylogenetic community structure. The apparent flexibility and additional benefits of the integrative ecophylogenetics framework can be applied to test hypotheses in a wide range of organisms, spatial, as well as phylogenetic scales. In the long term, this will deepen our understanding of how processes such as

environmental filtering, biotic interactions, or trait evolution interact over spatial and phylogenetic scales in governing species assembly into communities.

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Appendix A

We used generalized linear models (GLMs; Nelder and Wedderburn 1972), generalized additive models (GAMs; Hastie et al. 2009), boosted regression trees (GBMs; Friedman et al. 2000) and MaxEnt v. 3.3.3k (Phillips et al. 2006) to identify potentially suitable areas for Restionaceae species. We performed the modeling in R 2.15.1 (R Development Core Team 2012) with help of the 'gam' (Hastie 2011), 'gbm' (Ridgeway 2012) and 'dismo' (Hijmans et al. 2012) packages. The presences were weighted with a weight of one and the absences were weighted so that they reached, in sum, the same total weight as the presences. We restricted the number of trees in GBMs to 10,000, set the bag fraction to 0.5, and the maximal interaction depth to 3. We adjusted the learning rate ('shrinkage' option) to end up with 2000 to 8000 trees as the tree-set used for spatial projections. We used the same pseudo-absence data as for the other models as background data for MaxEnt and allowed only linear and quadratic features in order to avoid over-fitting. Increasing the convergence threshold to 0.0001 (default 0.00001) speeded up the model fitting process without reducing accuracy of MaxEnt model fit. We used eight model parameterizations that consisted of various combinations of bioclimatic variables from the WorldClim database (Hijmans et al., 2005) at a resolution of 30 arc seconds, as well as average annual radiation and potential evapotranspiration. We selected variable combinations to avoid collinearity by ensuring the variance inflation factors (VIF) of all selected variables within a parameterization were below five.

The parameterizations were defined as follows:

Parameterization	1	2	3	4	5	6	7	8
Mean Annual Temperature	X				X	X		
Mean Diurnal Range	X							
Isothermality						X		
Minimum Temperature of Coldest Month			X					X
Temperature Annual Range		X		X	X		X	
Annual Precipitation	X		X		X		X	
Precipitation Seasonality	X	X						
Precipitation of Warmest Quarter		X		X		X		X
Precipitation of Coldest Quarter				X		X		X
Potential Evapotranspiration		X	X	X			X	X
Radiation					X			

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Appendix B

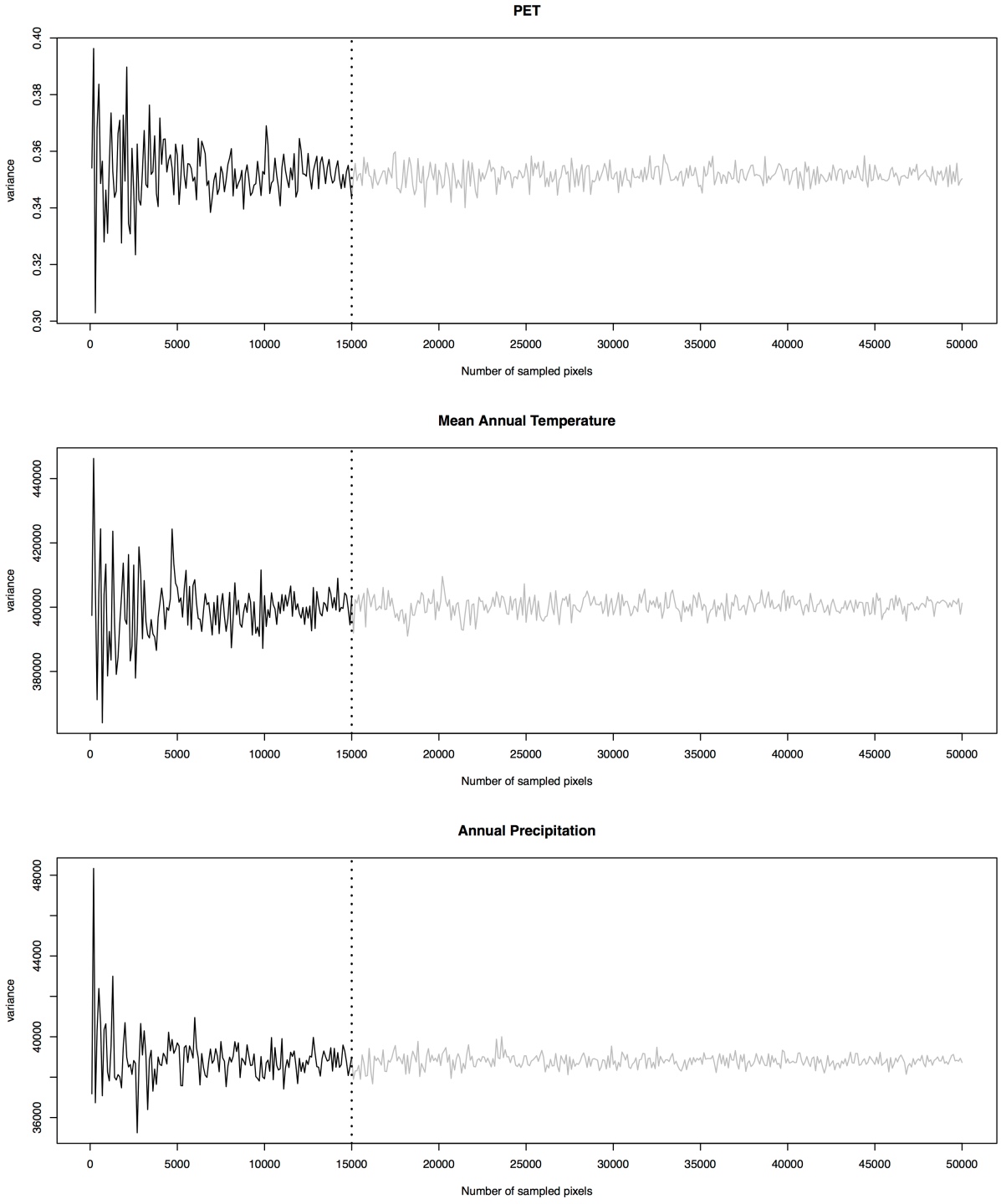

Figure A1 Variance in three climatic variables that represent major environmental gradients of the CFR. The variances are plotted against the number of sampled pixels throughout the CFR. The dashed line represents the number of pseudo-absences selected in the modeling procedure.

Appendix C

Table A1 Quartiles of species richness per QDS for each species pool.

	min	25%	50%	75%	max
sample	0	1	3	4	12
observations	0	1	6	20	316
models	0	8	32	70	196
vegtype	0	36	62.5	126	332
bioregions	0	168.5	347	348	349
biomes	0	348	348	349	349
biogeographic	6	85	160	335	349
global	349	349	349	349	349

Appendix D

Table A2 Random effects of species pools and phylogenetic scale on Intercept and Slope of NRI and NTI along PET. N indicates mean species richness per QDS in the case of species pools and clade richness in the case of phylogenetic scale.

	N	NRI	NTI
Species pool			
sample	3	-0.16	-0.19
observations	6	-0.11	-0.15
models	32	<0.01	-0.04
vegtype	63	0.02	<0.01
bioregions	347	0.05	0.10
biomes	348	0.05	0.10
biogeographic	160	0.09	0.09
global	349	0.05	0.10
Phylogenetic scale			
Restionaceae	110	-0.16	-0.09
Restioneae	93	0.16	0.09

CHAPTER V AVAILABLE CLIMATE REGIMES DRIVE NICHE DIVERSIFICATION IN GRASSE DURING RANGE EXPANSION

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We assess how inter-continental climate differences affected the occupancy and evolution of climate niches. Although the Danthonioideae (Poales) occupy temperate habitats on all continents and climatic niche evolution is phylogenetically constrained, we found significant niche differentiation between the continents. We also identified niche truncation along the major ecological gradients. Furthermore, we show that inter-continental differences in available climate predict inter-continental differences in selective regimes. These results indicate that three processes influence the expression and evolution of climatic niches: niche truncation, where the full potential range of the lineage cannot be expressed due to the regional absence of suitable climates; opportunistic evolution, where niches have shifted towards the available climatic space; and increased niche shifts associated with the colonization of new continents. These findings and the methodology developed here could improve predictions for invasive taxa and long-term responses to climate change.

Introduction

The modern diversity of plants is the result of their diversification into almost every habitat on earth, and consequently understanding the patterns and processes of niche diversification is central to understanding the evolution of ecological differentiation. In order to explore the genesis of present niche diversity, and also to predict future evolution of diversity, it is necessary to reconstruct the patterns, constraints and drivers of past evolutionary history. Here, we investigate a variety of processes to better understand observable patterns of niche occupancy and evolution.

Processes that affect the expression and evolution of niche occupancy may be intrinsic or extrinsic. Intrinsic constraints may be genetic (the possibility of evolving new structures or physiologies), functional (ensuring that the resulting organism is still functional), or developmental (what is ontogenetically possible; Smith et al. 1985). Extrinsic factors, in the form of the availability of habitats or co-occurring species, define the selective regime and so influence the evolution and expression of niches (Wiens et al. 2010).

Extrinsic factors are mainly driven by climate variability and competition among species, and by the novel environments a clade faces during geographic range expansion. Consequently, selective regimes can change through time, or through dispersal into new areas, and often influence niche evolution through new ecological opportunities, resulting in the occupation of new habitats or of new domains of the ecological gradient. Occupying new habitats may be possible because of, inter alia, enemy release (Henery et al. 2010), lack of competitors (e.g. Geospiza on the Galapagos; Schluter 2000), phenotypic plasticity (Riis et al. 2010), or adaptive evolution (Henery et al. 2010). Whatever the initial process is, it may result in a predictable, directional change in the niche occupancy of a dispersed clade. There is growing evidence from invasive species research that rapid evolutionary change is likely during a successful colonization (reviewed in Whitney and Gabler 2008, but see Trtikova et al. 2010). Such adaptive niche shifts during the colonization process of a lineage may lead to a punctuated evolution (Eldredge and Gould 1972). A different process happens if there is a lack of habitat (niche space) in the new area occupied by an expanding clade as compared to the source area. Here, the dispersing clade might not be able to find habitats encompassing the whole inherited potential niche breadth, resulting in a truncated realized niche (Gonzales et al. 2009, Feeley and Silman 2010).

A central concept in studies that assess intrinsic constraints is niche conservatism (NC), which reflects the tendency of closely related species to have (and maintain) similar niches (Wiens and Graham 2005, Wiens et al. 2010). However, the nature of niche conservatism is disputed. It has been shown to apply within species (Wiens and Graham 2005), to sister species comparisons (Peterson et al. 1999), among species within a clade (Bystriakova et al. 2011), among plant families within a continent (Prinzing et al. 2001), and among diverse plant clades in the southern Hemisphere (Crisp et al. 2009). NC appears not to be generally applicable, and for entire clades, containing a mix of sympatric and allopatric species, both niche divergence and convergence have been documented (Losos et al. 2003, Evans et al. 2009). To better understand how NC may influence the expression and evolution of niches, not only patterns should be examined, but also ecological, evolutionary and biogeographic processes that may lead to NC. This may be particularly important when using the concept of NC to predict biological responses to climate change or geographical expansion (Wiens and Graham 2005, Wiens et al. 2010). NC can be linked to niche occupancy, since the strength of phylogenetic constraints should allow us to predict whether species can react to changes in selective regimes. Niche parameters that are highly constrained phylogenetically should not adapt to changing environments as much as niche parameters that are phylogenetically less constrained.

The grass subfamily Danthonioideae is the ideal study group to explore patterns of niche occupancy and evolution on a global scale because of its global distribution in primarily temperate climates (Fig. 1a). Recent research has unravelled the phylogenetic (Pirie et al. 2008, Humphreys et al. 2011, Antonelli et al. 2011, Fig. 1b) and biogeographic (Linder and Barker 2005, Linder et al. 2013) history of the clade, the taxonomy has been clarified (Linder et al. 2010) and the ecological and distributional data are well documented for 281 species in 17 genera. Furthermore, the clade is large and old enough to provide good tests of NC during niche evolution. This is important as the scale at which NC is assessed may be a factor that influences outcomes of such analyses (Losos 2008). The Danthonioideae likely originated in southern Africa in the Oligocene (21-38 Ma; Bouchenak-Khelladi et al. 2010) and have since dispersed multiple times to finally occupy all continents except Antarctica and Greenland (Linder et al. 2013).

In this study we investigate how extrinsic drivers interact with intrinsic constraints to determine the evolution of the climatic niches of the grass subfamily Danthonioideae. First, we explore the co-variation between niche parameters and the phylogeny of the subfamily.

We compare whether this covariation is best explained by phylogeny alone or whether there is a spatial (continental) pattern as well, as has been found for the scaly tree ferns (Bystriakova et al. 2011). We then investigate whether the spatial pattern in the Danthonioideae could be the result of truncated niches, of opportunistic niche evolution, or of niche shifts associated with dispersal from one continent to another. We discuss the impact of the possible processes on the interpretation of current niche occupancy, the evolutionary history, and future development of the niches of globally distributed clades in a changing world.

Materials and Methods

Niches and Phylogeny

We collected 20800 occurrence points from herbaria that mainly represent the Southern Hemisphere and Asia (see Table S1 in Supporting Information). Additionally, we collected 27000 occurrences that cover North America and Europe from GBIF (gbif.org 2008; see Table S2). After removing species that were not in the phylogenetic tree, the database consisted of 209 species. We used ArcGIS 9.2 (ESRI 2008) to exclude unreliably georeferenced points and to overlay the occurrences with nineteen Worldclim bioclimatic layers (Hijmans et al. 2005) at a resolution of 30 arc seconds. In order to describe the ecological niche space available on each continent we sampled the terrestrial parts of the world with a lattice of 0.5° spacing on the same bioclimatic data layers. This provides a similarly sized data set (62000 points) as the combined Danthonioideae occurrence points. The areas we defined here (hereafter referred to as continents, see Fig. 1a) are Africa, Australia, New Zealand (including the Pacific Islands), South East Asia (including Papua New Guinea, Indonesia and the Philippines), Northern Asia, Europe, South America and North America (including Panama and countries further north). The taxonomy and nomenclature of the Danthonioideae follows Linder et al. (2010).

To reduce the nineteen climatic niche dimensions of the Danthonioideae we used the outlying mean index (OMI) ordination (Doledec et al. 2000) from the ade4 package (Dray and Dufour 2007) in R (R Development Core Team 2009). We preferred OMI over other ordination techniques because it does not assume any particular distribution of a species' response along environmental gradients and does not weight the observation site by species richness or abundance, assumptions which might not hold for intercontinental niche comparisons. From the transformed axes (OMI axes hereafter), we extracted the minimum, mean and maximum of the scores as niche parameters for each species. In order to account for possible

uncertainties resulting from the initial data (specimen misidentification, inaccurate georeferencing), we used the 5% and 95% quantiles as the minima and maxima. We obtained the parameters of the available climatic space on each of the continents in the same way, but without excluding the 5% quantiles on both extremes of the gradient because there are no identification and georeferencing errors with regards to continents.

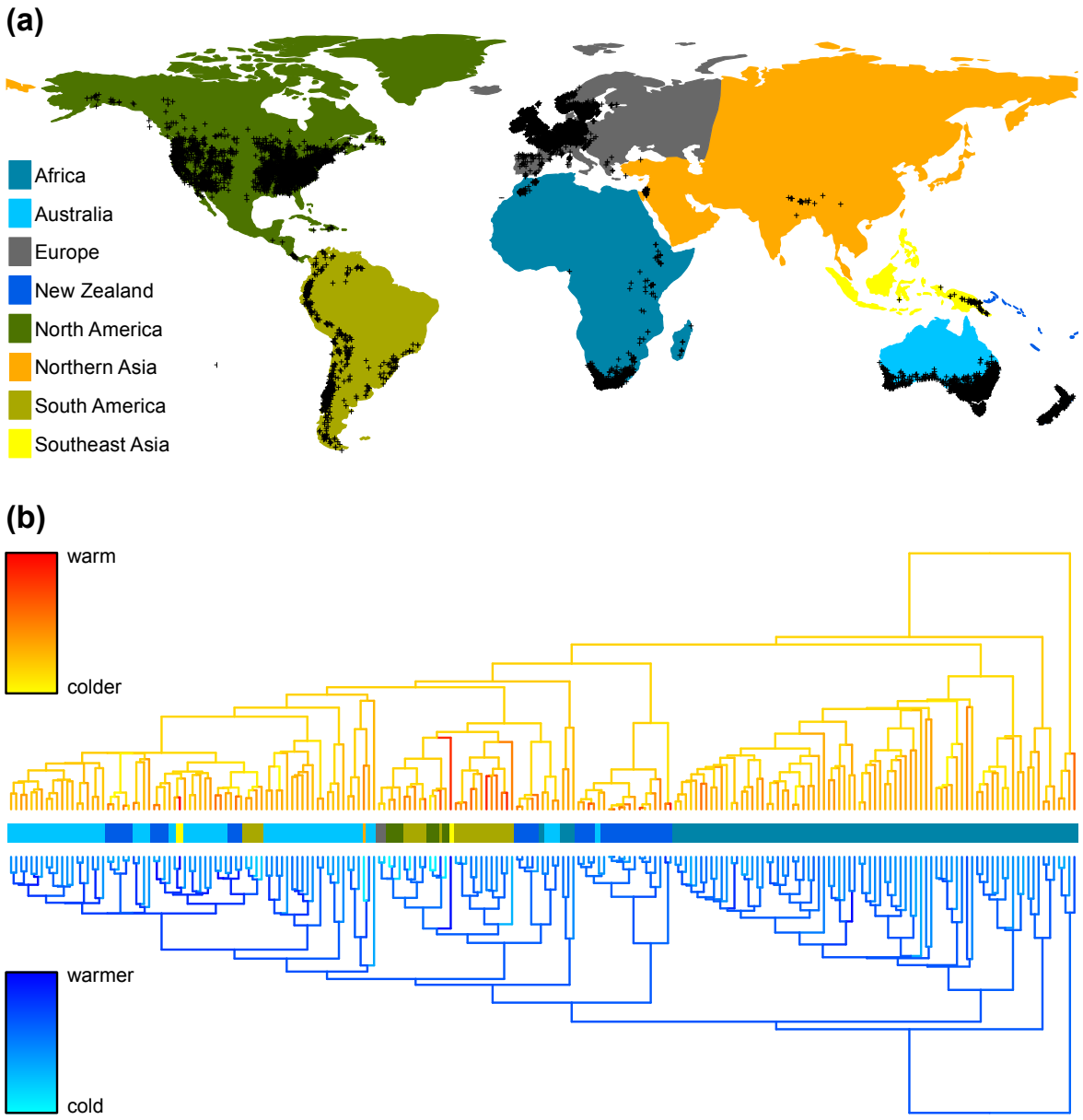

Fig. 1 Worldwide distribution of the mostly temperate grass subfamiliy Danthonioideae in (a). Black crosses represent the 48'000 collected occurrences. The colour coded continents indicate the sampled domains for the available niches. The coloured branches of the two trees in (b) represent the cold, seasonal (upper tree) and the warm, non-seasonal (bottom tree) range limits of the 209 danthonioid species along the temperature gradient according to the legend. The tips are represented by the colours that represent the continents, colour coding as indicated in (a).

The phylogeny of the Danthonioideae was based on five non-coding and three protein coding plastid markers and one non-coding as well as one coding nuclear marker, and included 81% of the species. Trees were generated using Bayesian and relaxed clock methods in BEAST (Drummond and Rambaut 2007), and calibrated using a secondary calibration point obtained from Bouchenak-Khelladi et al. (2010). Details of the tree generating and molecular dating methods are described in Antonelli et al. (see also Humphreys et al. 2011, 2011). In order to account for topological and dating uncertainty, we used a set of 1000 trees for all analyses, representing a regularly sampled set of post burn-in trees generated in BEAST. Since the phylogeny contains several significant conflicts between the nuclear and plastid genomes (see Pirie et al. 2008), only the sequences referring to the nuclear genome were retained in the dataset, and each species was represented by a single terminal node on the phylogeny.

Intercontinental Differences

In order to determine whether each continent constituted a different selective optimum, we used the Ornstein-Uhlenbeck (OU) model of evolution that can estimate several selective optima (adaptive zones) for a given dataset (Butler and King 2004). We fitted OU models by assuming a different selective optimum on each of the eight continents (OU-8) and contrasted this model to one with a single, global selective optimum (OU-1). These models were then contrasted to Brownian Motion (BM) models and model fit for the different models was assessed from sample size corrected AIC.

We employed linear regressions to test whether the differences in available niche space on each continent can explain the different selective optima among the continents. For each continent we calculated the difference of the OU-8 selective optima and the clade-wide OU-1 selective optimum. We used these differences as the dependent variable, and the differences in available climatic space, calculated as the differences of the mean on each continent and the global mean, as the independent variable. In this way we tested whether danthonioid climate niches drifted away from ancestral niches, tending towards the climatic space available on each continent.

In contrast to Feeley and Silman (2010) who assumed that all species should have the same niche breadth, we test whether niche truncation has occurred by comparing the occupied and available range limits on each continent with danthonioid ranges globally, because this may be more appropriate when analysing multispecies clades. If the available range on a continent represents only a part of the range occupied by the danthonioids globally, it is possible that

the niche on that continent is truncated. This test is based on the assumption that the globally observed ecological range for the entire subfamily represents their fundamental niche (i.e. the entire realm of conditions where members of the danthonioid clade may survive and successfully reproduce).

Niche Evolution

Several methods exist to assess the correlation between interspecific ecological similarity and phylogenetic relatedness (i.e. phylogenetic signal), one common way of testing NC (Cooper et al. 2010). In order to test whether there is phylogenetic signal (PS) in the danthonioid climatic niches, we used Pagel's λ (Pagel 1999). This is a multiplier of the off-diagonal elements in a phylogenetic variance-covariance matrix that can be optimized in a maximum likelihood (ML) framework to best fit the observed tip data. Values range from zero to one and indicate whether the evolution of traits varies perfectly with phylogeny, equivalent to BM evolution (λ=1) or alternatively is completely independent from the phylogeny (λ=0).

To test whether danthonioid evolution was gradual or punctuated we used Pagel's κ (Pagel 1997). κ is a branch length scaling parameter that raises branch lengths to the power of κ, which means that a value of κ=1 can be interpreted as gradual evolution (traits change linearly with branch lengths) and κ=0 indicates punctuated equilibrium evolution. We used the 'fitContinuous' function of the 'geiger' package (Harmon et al. 2009) in R to calculate λ and κ and a Chi-square test to assess whether observed κ (or λ, respectively) are significantly different from 0 and 1.

We tested the hypothesis that niche shifts coinciding with long distance dispersal (LDD) events resulting in occupation of new continents are greater than those where no LDD occurred. We reconstructed the ancestral niche states (of the first two OMI axes) at each node on the set of 1000 trees, using ML. Ancestral areas were reconstructed using parsimony. Both procedures are available in the 'ace' function of the R-package 'ape' (Paradis et al. 2004). The significance of the differences, as well as the rate shifts (absolute differences divided by branch length), between nodes where intercontinental dispersal occurred, compared to those without dispersal, were assessed using a non-parametric Wilcoxon-Rank test.

Results

Niche and Climate Description

The cumulative inertia (cumulative variance, see Table S2) of the axes resulting from the OMI-analysis showed that the first two axes explain 47% and 27% of the total variance respectively (74% in total), whereas each of the remaining axes explained less than 10%. We therefore only used the first two axes for further analyses. OMI axis 1 was mainly related to temperature variables and indicated cold temperatures with high seasonality (arctic-alpine climate) at the lower (minimum) axis end and warm temperatures with low seasonality (tropical climate) at the upper (maximum) axis end (see Table S3). Hereafter we refer to OMI axis 1 as the temperature axis ranging from cold seasonal to warm aseasonal conditions. Fig. 1b shows how both the cold and warm range limits of this axis are distributed on the phylogeny. OMI axis 2 can be interpreted as a seasonal aridity gradient, indicating all-year wet conditions at the upper axis end and seasonally arid conditions at the lower axis end. This is substantiated by positive correlations of OMI axis 2 with annual precipitation, precipitation of both the driest month and quarter and with negative correlations with precipitation seasonality (see Table S3). Consequently we refer to this as the rainfall axis, ranging from seasonally dry to wet conditions.

There were clear differences in the available climatic space as well as the occupied niches on the different continents. Northern Asia, South- and North America had the widest range of climatic conditions (Fig. 2) whereas Australia and Europe had the most restricted available climatic space. However, there was also substantial overlap in the breadth of available climate space (minimum to maximum, e.g. Northern Asia and North America, New Zealand and Australia, or Africa and South America). Overlap of the core of available climate (filled boxes in boxplots, corresponding to 50% around the median) compared to the climatic range that is occupied by the danthonioids on the temperature axis was very low in general (with the exception of New Zealand). This pattern was different for the rainfall axis: on four out of eight continents the core of the danthonioid niche was totally enclosed in the core of the available niche space. Only the core of Africa and Australia did not at all overlap with the core of available niche space.

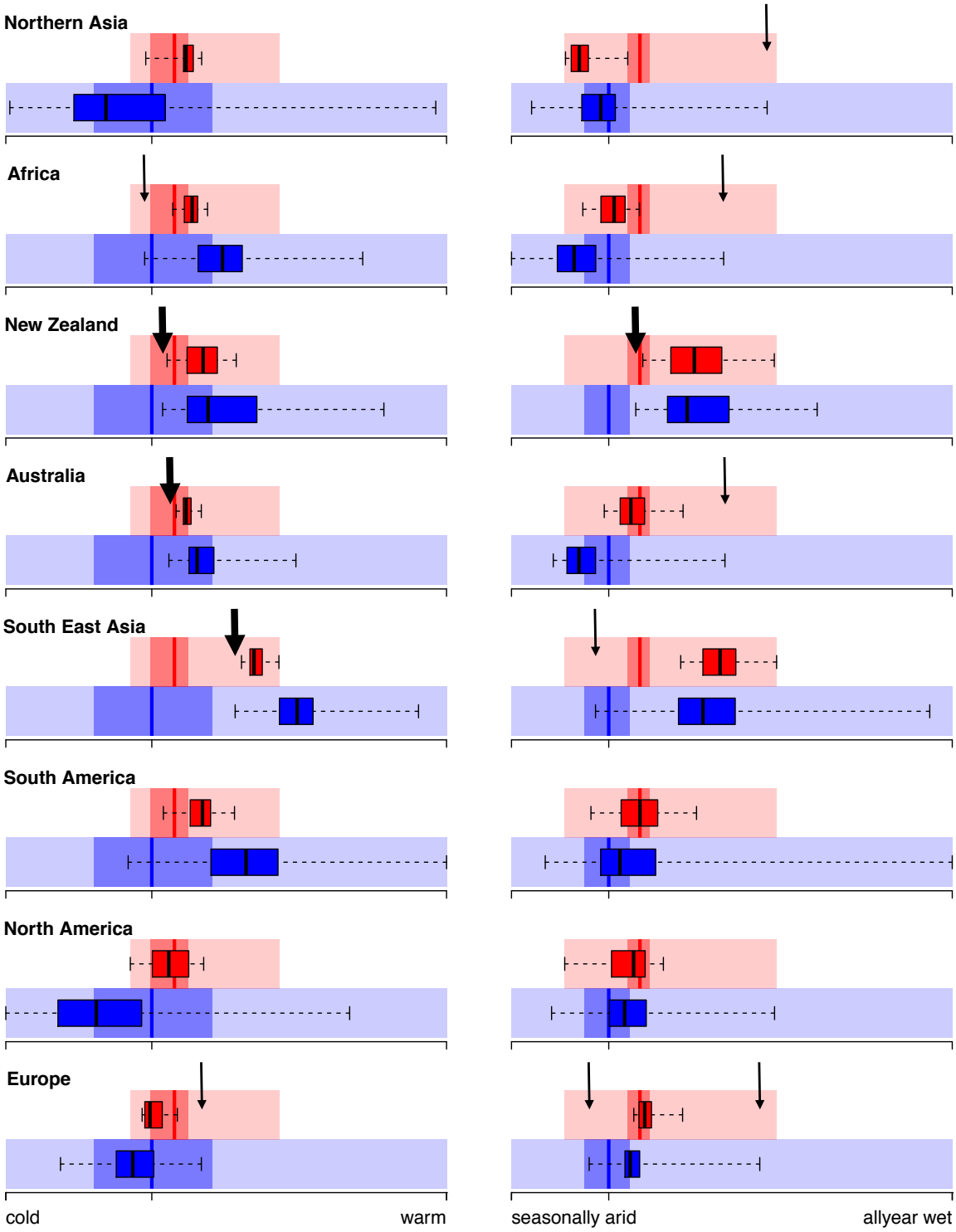

Fig. 2 Representation of the niches per continent, temperature axis in the left and precipitation axis in the right panel. Red colours represent occupied niches, blue colours represent available niche space. The light background colours indicate the global range (min-max) of available climate space, with the quartiles and median slightly darker. The embedded box plots report the quartiles of the occupied range for each relevant continent, the range of which is indicated by the stippled line. The arrows indicate putative cases of niche truncation (see Discussion). The bold arrows indicate the most likely cases of niche truncation where the limits of the occupied niche closely approach the limits of available climate.

Intercontinental Differences

The AICc value for every OU-8 model was considerably lower than the AICc values for both BM and OU-1 models (Table 1), indicating that different selective regimes acted on the danthonioid clades on the different continents resulting in adjusted optima. The continental values of the selective optima are displayed in Figure S2.

The available climate space on each continent largely predicted the intercontinental deviations in the selective optima along the niche axes of the danthonioids (Table 2). The regressions on the temperature axis were all highly significant ($p<0.003$) with very high R2 (0.758-0.943), whereas on the rainfall axis the p-values ranged from 0.023-0.074 (only the minima were not significant) with generally lower R2 values.

There are two indications of niche truncation. The first is when the occupied niche of the entire Danthonioideae subfamily exceeds the available climate space on a continent (arrows in Fig. 2). This was evident for the warm range edge along the temperature axis in Europe, and

Table 1 AICc values of the three different evolutionary models Brownian Motion (BM), Ornstein-Uhlenbeck with one global optimum (OU-1) and Ornstein-Uhlenbeck with eight optima (one optimum per continent OU-8). The reported values represent the range of AICc values obtained from fitting the evolutionary models on a set of 1000 time-calibrated trees, with the best model in bold.

	BM	OU-1	OU-8
	(min) mean (max)	(min) mean (max)	(min) mean (max)
Temperature axis			
cold, seasonal	(542.7) 698.5 (1510.0)	(474.0) 494.9 (501.3)	(383.2) **393.2** (395.4)
mean	(548.6) 700.5 (1453.0)	(452.4) 474.0 (478.3)	(365.6) **371.6** (372.0)
warm, non-seasonal	(705.4) 838.0 (1435.0)	(604.6) 618.4 (619.9)	(516.6) **523.1** (547.7)
Rainfall axis			
seasonally arid	(705.9) 859.3 (1583.0)	(691.2) 772.0 (866.9)	(639.8) **690.5** (949.1)
mean	(766.7) 860.6 (1508.0)	(751.6) 809.3 (1061.0)	(685.3) **734.0** (775.2)
all-year wet	(987.1) 1070.0 (1551.0)	(944.2) 985.9 (1050.0)	(861.1) **877.0** (886.3)

Table 2 Explained variances (R^2) and signify-cance (p) of the regression of intercontinental deviations in the selective optima from the OU-8 models explained by available climate space.

	R^2	p
Temperature axis		
cold, seasonal	0.76	0.003
mean	0.94	<0.001
warm, non-seasonal	0.91	<0.001
Precipitation axis		
seasonally arid	0.36	0.074
mean	0.48	0.035
all-year wet	0.54	0.023

for the cold range edge in Africa, New Zealand, Australia and SE Asia; and along the rainfall axis for the seasonally arid range edge in New Zealand, SE Asia and Europe, and for the all-year wet range edge in N Asia, Africa, Australia and Europe. The second indication arises if, in addition, the occupied range on a continent approaches the same limit as the available range (bold arrows in Fig. 2). This makes niche truncation even more probable and was evident for the temperature axis for the cold limit in New Zealand, Australia, and South-East Asia (thus cold temperatures with maximum seasonality). In addition our results indicated that the rainfall axis might also be truncated at the dry end in New Zealand (seasonally arid conditions). In these cases the two central quartiles of the occupied niche were also shifted away from the two central quartiles of the globally occupied niche.

Niche Evolution

For the evolution of climate niches, as quantified by the minimum, mean and maximum values retrieved from the rainfall axis, as well as the warm end of the temperature axis, we could reject the null hypothesis of no phylogenetic signal according to Pagel's λ (0.24-0.93, see Table 3). The hypothesis of niche conservatism was also rejected, as Pagel's λ differed significantly from 1 (Table 3). For the mean and minimum (cold) values derived from the

temperature axis the hypothesis of niche conservatism was likewise rejected ($0.05<\lambda<0.66$), but the majority of topological replicates (93% of the mean and 78% of the maximum) did not reject the null hypothesis of no phylogenetic signal. This showed that while there was PS in the majority of the investigated niche parameters, there was no support for evolution following BM for either temperature or precipitation.

Table 3 Pagel's λ and κ of the six niche parameters derived from the temperature and the precipitation axis. The ranges (min-max) stem from estimating all niche parameters on a set of 1000 time-calibrated trees. The columns λ vs. 0 (λ vs. 1, respectively) indicate the percentage of trees for which we detected a phylogenetic signal that is significantly different from 0 (1, respectively) at a significance level of 0.05. The columns κ vs. 0 and κ vs. 1 report the analogous percentages for Pagel's κ.

	Pagel's λ	λ vs. 0	λ vs. 1	Pagel's κ	κ vs. 0	κ vs. 1
	(min) mean (max)			(min) mean (max)		
Temperature axis						
cold, seasonal	(0.052) 0.223 (0.660)	21.7 %	100.0 %	(<0.001) 0.075 (0.584)	15.8 %	100.0 %
mean	(0.047) 0.184 (0.606)	7.5 %	100.0 %	(<0.001) 0.022 (0.367)	4.8 %	100.0 %
warm, non-seasonal	(0.244) 0.347 (0.439)	100.0 %	100.0 %	(<0.001) 0.001 (0.138)	0.0 %	100.0 %
Rainfall axis						
seasonally arid	(0.783) 0.840 (0.925)	100.0 %	100.0 %	(<0.001) 0.050 (0.348)	3.3 %	100.0 %
mean	(0.806) 0.868 (0.933)	100.0 %	100.0 %	(<0.001) 0.117 (0.370)	12.3 %	100.0 %
all-year wet	(0.641) 0.709 (0.774)	100.0 %	100.0 %	(<0.001) 0.005 (0.186)	0.0 %	100.0 %

Pagel's κ was generally very low (see Table 3). In every case it was significantly different from $\kappa=1$ and with few exceptions it was not significantly different from $\kappa=0$. The exceptions in the seasonally arid range edge (minimum along precipitation axis) are <5%, which is to be expected at a significance level of 0.05. The proportion of significant topological replicates in the temperature minimum (16%) and the precipitation mean (12%) are still very low and consequently we cannot reject the hypothesis of punctuated niche evolution in the danthonioids.

There was no significant difference between the magnitudes of niche evolution rate shifts at nodes with dispersal between continents compared to rate shifts at nodes without intercontinental dispersal. However, using branch length information to infer rate shifts may not be appropriate in this case, as κ indicated that niche evolution in the danthonioids was punctuated. We observed a trend (see Figure S1) indicating higher absolute differences in niche parameters between ancestor and descendent nodes with dispersal between continents, than those without intercontinental dispersal. For example, 53% (42%) of the 1000 trees showed a significantly greater niche shift in the limits towards cold, seasonal temperatures (and towards all-year wet conditions respectively) between dispersal and non-dispersal nodes. Estimating ancestral states of continuous characters is generally inaccurate (Webster and Purvis 2002) and may smoothen ancestor-descendent values, which will reduce the differences between dispersal and non-dispersal nodes. Our results, therefore, likely underestimated the significance of these differences.

Discussion

We show that the climatic niches of the danthonioids differ between the continents along both the temperature and the precipitation gradient. We consider three processes that could lead to the observed geographical patterns. Firstly, the differences in observed niche space may be partially the consequence of truncation of the fundamental niches due to the lack of available climatic space on some continents. Secondly, these differences could result from opportunistic niche evolution subsequent to invasion of new continents, where evolving species adapt to the available habitats on the newly occupied continents as a response to shifts in selective regimes. If the latter applies, then it is possible that most niche evolution occurs during or following the third process: intercontinental dispersal. All three processes suggest that extrinsic factors have played an important role in both niche expression and niche evolution in the danthonioids. Phylogenetic constraints, as an intrinsic counterpart to the extrinsic drivers may not only hinder the danthonioids from fully exploring the entire range of globally available climatic conditions but also limit the magnitude of an adaptive response to the extrinsic drivers.

Niche Truncation

Numerous examples indicate truncated niches in the danthonioids. Niche truncation may occur if the danthonioids reach the limit of available climatic space on a continent, even though their potential is wider (Fig. 2). This is evident for the cold and seasonal limits in New

Zealand, Australia and South East Asia, as well as for the range limit towards seasonally arid conditions in New Zealand. The same finding is also corroborated by experimental evidence showing that intrinsic cold tolerance is indeed wider than suggested by the realized niche (Humphreys and Linder, personal communication). Two processes could result in truncated niches. Firstly, a lineage might disperse to a continent that contains only part of its potential climatic range. Alternatively, climate change could alter available conditions and thus lead to loss of suitable climates. New Zealand presents an excellent example for both processes. Most of the rainfall gradient occupied by the danthonioids in the ancestral area (Africa), especially seasonally arid habitats, is not available in New Zealand and consequently the rainfall axis may be truncated at the dry end. In addition, New Zealand's temperature axis may have been truncated at the cold end by climate change following the last glacial maximum. However, only experiments will allow testing whether New Zealand species can indeed tolerate drier and colder conditions.

Generally, truncated niches, at least at the supra-specific level, might be common. There are various consequences of not taking niche truncation into account in evolutionary biology and ecology. Firstly, underestimation of niche breadth due to truncation of available habitat could lead to the false conclusion that niche evolution has occurred. Secondly, when applied to potential invasive species, it could result in the underestimation of the potential ranges of neophytes, which may have happened in the numerous examples of invasive species that occupy new habitats in the invaded area (Broennimann et al. 2007, Medley 2010). Thus, the potential presence of niche truncation, and truncation of available climate space should be considered when comparing native and invaded niches (Peterson and Nakazawa 2007, Mandle et al. 2010). Thirdly, niche truncation in the current ranges of species may lead to the underestimation of the potential habitat of species following global change, and so may lead to incorrect predictions of responses to climate change or invasions (Feeley and Silman 2010).

Opportunistic Evolution

Evolutionary adaptations to newly available conditions have been demonstrated to occur after invasions (Henery et al. 2010), and our results are best interpreted to show climatic adaptation in the Danthonioideae. AICc is always better when assuming eight different optima in Ornstein-Uhlenbeck models than when assuming a single optimum (Table 1), suggesting that there are different selective regimes acting on the different continents. This interpretation is corroborated by preliminary experiments indicating that observed range limits can serve as a relative proxy for fundamental, physiological limits in the Danthonioideae (Humphreys &

Linder, personal communication). The significant relationship between the intercontinental shifts in the selective optima and the corresponding shifts in available climate space (Table 2) is consistent with this interpretation. The pattern holds for almost all niche parameters (minimum, mean, maximum) on both axes. It is also evident that the less phylogenetically constrained a niche parameter is, the easier it is to predict intercontinental deviances in OU optima from the available climate on a continent. We interpret this as a strong signal that available climate space is influencing niche evolution in a probabilistic manner during species diversification.

Intercontinental Dispersal

We postulate that danthonioid niches evolved most strongly in association with intercontinental dispersal events. The statistical evidence for this is weak, but ancestral state optimization methods may obscure the pattern by assuming gradual change along branches (Webster and Purvis 2002), which is not likely given the very low values of Pagel's κ. Furthermore, the low number of dispersed compared to non-dispersed nodes (<1:10, less than 20 dispersals out of more than 200 speciation events) reduces the strength of the pattern. The evidence for punctuated evolution in the clade is also consistent with niche shifts associated with few nodes. The occurrence of PS is also consistent with this pattern, as there are relatively few dispersal events, resulting in clades of species that are geographically restricted to one continent, and which all share a similar niche. The evidence of rapid niche evolution associated with continent change from recent invaders is still contentious and there is as yet no convincing evidence from deeper time.

Phylogenetic constraints

Niche evolution in the danthonioids shows considerable PS, but no niche conservatism sensu Losos (2008) or Cooper et al. (2010). A significant phylogenetic signal, such as obtained for maximum temperature and for seasonal water availability (rainfall axis), appears easy to interpret as it follows as a consequence of descent with modification. PS in the maximum temperature indicates that the evolution of species' tolerances towards warm, non-seasonal climates varies in accordance with the phylogeny, which is consistent with the current restriction of danthonioids to temperate climates. However, the reason for this restriction is not clear. It could be the result of physiological constraints, or competitive displacement by the more water efficient C4 grasses in hotter climates (Osborne and Freckleton 2009). This is similar to phylogenetic niche conservatism reported in the Appalachian Salamanders,

preventing their dispersal into the warmer and drier lowland conditions (Kozak and Wiens 2006, 2010). The hypothesis of an absolute physiological limit, which in effect constitutes a fundamental niche parameter keeping the danthonioid clade out of tropical areas, is amenable to experimental testing.

It is difficult to interpret the failure to reject hypotheses of no PS. Our failure to reject the hypothesis of no PS at the cold limit of danthonioids is unexpected, as cold-tolerance is often understood as being conserved (Wiens and Donoghue 2004), with only few tropical clades successfully adapting to frost. One explanation is that very low rates of evolution (phylogenetic inertia) lead to a reduction of the phylogenetic signal (Revell et al. 2008). For the danthonioids this would imply that phylogenetic inertia (i.e. niche conservatism) for the cold, seasonal climate is so strong that it reduces measures of phylogenetic signal. Another explanation is that the evolution of cold tolerance is phylogenetically unconstrained (contrary to the expectation above), and that every genus in the Danthonioideae includes species from both warm and cold habitats. This could be due either to the state being labile (i.e. changing readily, as e.g. in Oenothera; Evans et al. 2009) or because the tolerance of each species is very wide (not a constraining parameter). The standard deviation for the cold, seasonal limit per genus in the Danthonioideae is significantly larger than that for the warm, non-seasonal limit, indicating that tolerances to cold and seasonal climate are evolutionarily labile. It is possible that the critical, conservative element in cold tolerance is whether species tolerate frost. However, if the whole subfamily is cold tolerant, as implied by (Edwards and Smith 2010), then the degree of cold stress (e.g. the number of consecutive days with frost) that can be tolerated might be evolutionary labile among closely related taxa (Humphreys & Linder, in revision). Thus the finding of no PS is probably due to too much change (the niche parameter being evolutionary labile) rather than too little (phylogenetic inertia).

We find that all variables of the rainfall axis, and the minimum of the temperature axis, show PS, while the maximum and mean of the temperature axis do not. The commonly used approach (Blomberg et al. 2003, Kozak and Wiens 2010, e.g. Bystriakova et al. 2011) of analysing only trait means would have led to the conclusion that the temperature axis is not conserved. This suggests that focussing only on mean trait values for each species could be misleading. Evans et al. (Evans et al. 2009) suggested sampling the niche parameter distribution per species. This arguably provides a better estimate of the distribution, but is not useful when the upper or lower limits of the variation themselves are more informative than the mean trend. Analysing minima and maxima may also help to interpret results from NC

analyses since the minimum along an ecological gradient may reflect different physiological adaptations than the maximum.

Conclusions

The analysis of the patterns of niche evolution in the danthonioid grasses shows phylogenetic signal with geographic structure. We demonstrate that this geographic structure is partially the result of truncated niches (temperature) and partially of niche evolution strongly drifting towards the most common climate habitats available on different continents (precipitation). This may lead to rapid niche shifts following the occupation of a new continent. We do not know from correlative approaches what unexpressed potential the studied species might have to occupy unavailable habitats, and thus we do not know their adaptive potential well. Answering these questions will only be possible with the help of experiments. However, knowing the range of possible ecological and evolutionary responses when confronted with new environments allows us to better predict how clades may respond to global change in the longer term.

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Supporting Information

Table S1 List of Herbaria we obtained occurrence data from (Southern Hemisphere).

AK	Auckland War Memorial Museum Herbarium, New Zealand
BOL	University of Cape Town Bolus Herbarium, South Africa
BRI	Queensland Herbarium, Australia
CANB	Australian National Herbarium, Australia
CHR	Allan Herbarium, New Zealand
CONC	Universidad de Concepción Herbario, Chile
GRA	Albany Museum Selmar Schonland Herbarium, South Africa
HO	Tasmanian Museum & Art Gallery Herbarium, Australia
K	Royal Botanic Gardens Herbarium, United Kingdom
MEL	Royal Botanic Gardens National Herbarium of Victoria, Australia
MO	Missouri Botanical Garden Herbarium, United States
NBG	South African National Biodiversity Institute Compton Herbarium, South Africa
NSW	Royal Botanic Gardens National Herbarium of New South Wales, Australia
PERTH	Western Australian Herbarium, Australia
PRE	National Botanical Institute National Herbarium, South Africa
Z	Universität Zürich Herbarium, Switzerland
ZT	Eidgenössische Technische Hochschule Zürich Herbarium, Swizterland

Table S2 This supplementary table contains a list of all GBIF accessions, is not included in this thesis, but can be obtained from the author upon request.

Table S3 Inertia (variance) of the first three axes of the OMI ordination.

Axis	Eigenvector	Inertia	Cumulative Inertia
1	8.9305307747	0.4700279	0.4700279
2	5.1773478727	0.2724920	0.7425199
3	1.0520072037	0.0984558	0.8409755

Table S4 Canonical correlation of initial bioclimatic variables with the axes resulting from the OMI analysis.

Initial variable	OMI axis 1	OMI axis 2	OMI axis 3
bio1	0.936435391	-0.287040325	0.038000085
bio2	0.297508641	-0.72085594	0.023992371
bio3	0.8921383	-0.07416968	-0.141367826
bio4	-0.897115941	-0.17055938	-0.075892647
bio5	0.676677926	-0.610097894	0.030773212
bio6	0.96516041	-0.046790202	0.080266671
bio7	-0.805515415	-0.363541498	-0.084774565
bio8	0.594755031	-0.493935352	-0.177301341
bio9	0.903976829	-0.137501663	0.156481723
bio10	0.745461494	-0.528179744	0.015940445
bio11	0.969303444	-0.133064496	0.060459872
bio12	0.589626992	0.66146485	-0.709146701
bio13	0.612872852	0.339386627	-0.779724809
bio14	0.28121481	0.856538129	-0.358938554
bio15	0.155370662	-0.700675093	-0.214208738
bio16	0.612992034	0.390133148	-0.779141094
bio17	0.31011579	0.856042349	-0.389589472
bio18	0.360939041	0.487659091	-0.776195835
bio19	0.467374835	0.651519322	-0.391398111

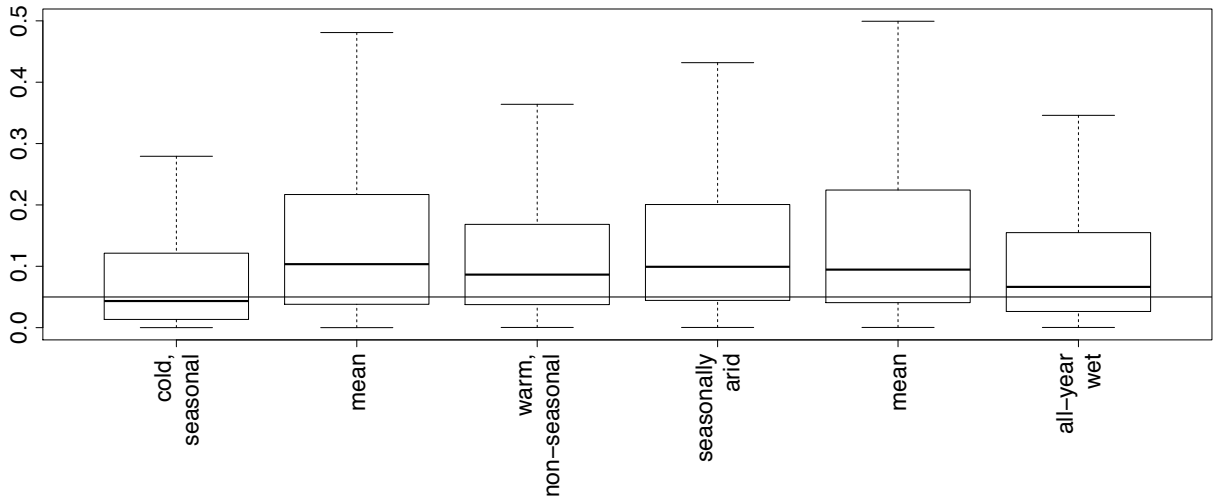

Fig. S1 Distribution of p-values from Wilcoxon Rank test that compared niche shifts at intercontinental dispersal with non-dispersal shifts. The test was applied to the entire set of 1000 time-calibrated trees. The horizontal line indicates the significance threshold of 0.05.

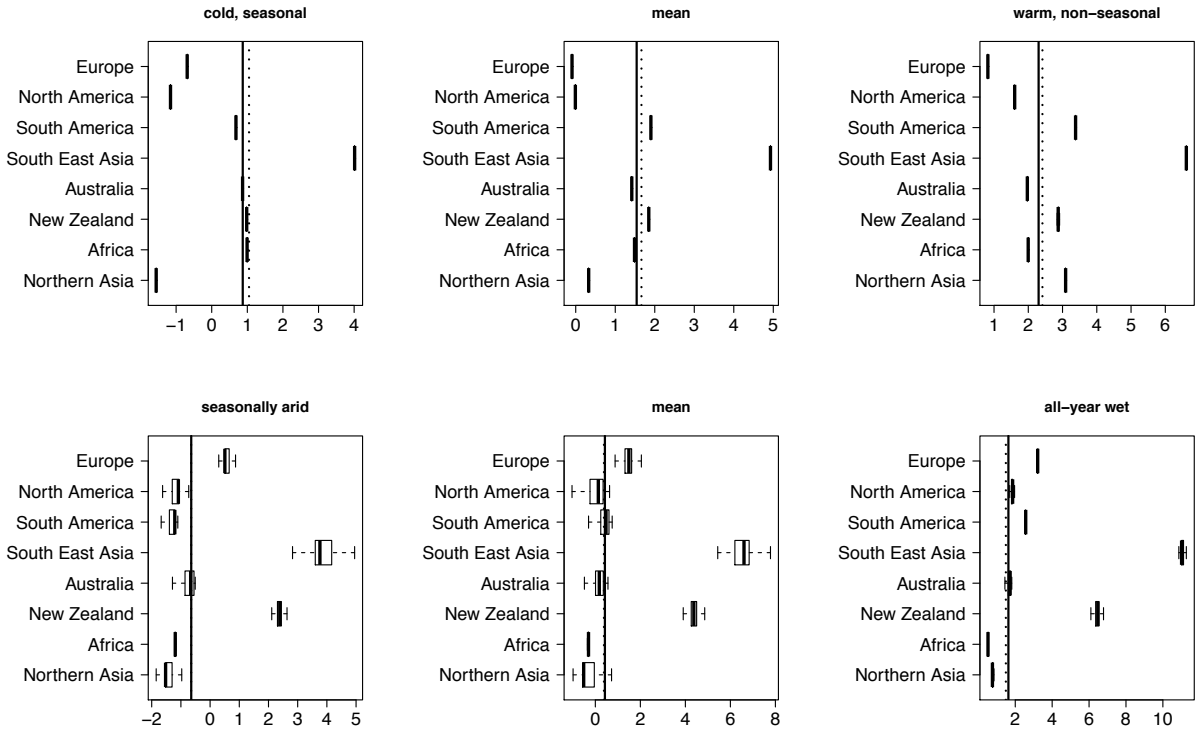

Fig. S2 The optimized selective optima from the OU-8 model (boxplots) are compared to the global selective optima from the OU-1 (solid line) and the BM model (dashed line). Note that AICc on every one of the analysed 1000 trees is best for OU-8 models, and better for OU-1 than for BM models.

CHAPTER VI RESEARCH OUTLOOK

RAFAEL O. WÜEST

This Chapter contains a collection of ideas on possible future research. These ideas arose during the work on the chapters of this thesis, during conferences and workshops, or during discussions with my supervisors and collaborators. The ideas should be taken as what they are: roughly outlined thoughts, which may serve as seeds that initiate thinking and discussion. All ideas need to further mature and develop to finally serve as starting points for actual research questions or projects.

Species Assembly in Replicated Restionaceae Radiations

Results in Chapter II indicate that reseeder-resprouter ratio in Restionaceae assemblages varies with climate and soil type, and that observed ratios in specific climates and soil types contradicts observed reseeder-resprouter ratios of other taxa in the CFR. The Restionaceae, thus, appear to be somewhat exceptional. A study that repeats the local assemblage sampling in the replicated Restionaceae radiation of Australia and New Zealand could resolve whether the observed pattern holds for the entire Restionaceae or whether it is a peculiarity of the Cape Restionaceae. Differences in the evolutionary trajectories of climate niche preferences between the two radiations (Litsios et al. submitted, see Chapter III) could indicate radiation-specific relations of reseeder-resprouter ratio towards environmental conditions.

Fire and Species Assembly

The studies on Restionaceae assembly in the CFR presented in Chapters II and IV miss one important aspect: fire. Fire is an important factor for the functioning of ecosystems (e.g. Bond and van Wilgen 1996, Bond et al. 2005). For example, resprouters are expected to be favored in frequently burnt vegetation (e.g. Bond and Midgley 2001, Vila-Cabrera et al. 2008, Cabral and Schurr 2010). Currently, the lack of a comprehensive, spatially explicit model of fire frequencies for the CFR did not allow me to incorporate fire frequency into the analyses. However, a recent fire model based on data from nature reserves (Wilson et al. 2010) is potentially well suited to extrapolate fire frequencies to the entire CFR. Such an extrapolation would reflect average natural fire frequencies in relation to climate and could be used to test whether resprouters are indeed favored in frequently burnt areas, i.e. whether fire frequency is negatively correlated with reseeder-resprouter ratio in Restionaceae assemblages.

Climate Change as Natural Experiment

A CFR wide fire model would also enable evaluating indirect effects of climate change, through altered fire regimes, on community composition. The fire model is driven by climate variables and as such could incorporate changes in temperature and precipitation as projected by climate models. Wilson et al. (2010) inferred a climate-driven increase in historical fire frequency: fires returned on average 4 years earlier in the period 1975-2000 compared to the period of 1951-1975, with substantial spatial variation. Spatial variation is also apparent in climate models, projecting higher temperature and precipitation seasonality (the main drivers of the fire model) in some areas, but not in others (Hewitson and Crane 2006, MacKellar et al.

2007). This spatial variation could serve as basis to set up a long-term observation study that exploits climate change as a natural experiment. Sampling design, i.e. the selection of observation sites, should thereby rely on current and future fire probability maps, including plots in low and high fire frequency areas, in areas with likely low and high changes in predicted fire frequencies, as well as in combinations thereof. One aspect that could be investigated is whether reseeder-resprouter ratio is indeed highest in plots with low historical fire frequencies, and whether reseeder-resprouter ratio will decrease in areas that actually experience high future fire frequencies. Better knowledge on how community composition is affected by fire frequency is needed in times of changing climate because fire management is an important aspect in biodiversity conservation (e.g. Seydack et al. 2007, Abbott and Le Maitre 2009, Van Wilgen et al. 2010).

The Integrative Ecophylogenetics Framework

The integrative ecophylogenetics framework that I introduced in Chapter IV offers ample opportunity for further research. The framework owes its flexibility to the incorporated mixed effects model, which allows to simultaneously evaluate effects of multiple factors on the phylogenetic structure of communities. I outlined in Chapter IV how various additional environmental variables (e.g. soil hydrology or environmental heterogeneity) potentially contribute to the scatter around estimated phylogenetic structure of Restionaceae assemblages along the moisture balance gradient. Future studies could include these variables, which should enlarge the proportion of explained variation in the models. A more thorough assessment of phylogenetic scale, which in Chapter IV was only assessed by comparing two scales, could reveal whether the observed increase in clustering at the smaller phylogenetic scale holds if additional scales are added, which appears to be important given that the observed results contradict previous work (Slingsby and Verboom 2006, Cavender-Bares et al. 2006, Vamosi et al. 2009).

The integrative ecophylogenetics framework is perfectly suited to be applied to a wide range of additional taxa. On one hand, reiterating analyses of phylogenetic community structure along environmental gradients in diverse groups of taxa could evaluate whether observed patterns in Restionaceae generally hold. For example, does the effect of phylogenetic scale generally dominate the effect of species pool definition? The flexibility of the framework also allows to investigate temporal changes in the phylogenetic structure of communities. Possible applications could include tracking the phylogenetic structure of disturbed vegetation

throughout succession. Following migratory individuals through life stages (e.g. salmonid fishes) and measuring the phylogenetic structure of communities that each successive life stage inhabits could be another possibility. Such studies would allow assessing whether general time-related trends emerge. Evaluating how phylogenetic structure in species assemblages varies through time is a largely neglected question in current research (but see Silver et al. 2012).

Eco-Evolutionary Feedbacks

Analyses as presented in Chapters II and IV help understanding how evolutionary processes affect structure within and among local species assemblages. However, feedbacks in the other direction, i.e. from coexisting species to evolutionary processes, are expected to take place, too. For example, historical range overlap of Biaka Western Pygmies in west central Africa with a chimpanzee subspecies that carries the simian immunodeficiency virus (SIV, ancestral to the pandemic human immunodeficiency virus HIV) lead to selection at several loci that are associated with resistance to HIV (Zhao et al. 2012). The emergence of obligate mutualism between clownfishes and sea anemones that lead to an ecological adaptive radiation is another example (Litsios et al. 2012, see APPENDIX III). The effect of particular coexistence patterns on evolutionary processes is so far only tested in case studies. Evaluating the generality of such phenomena and estimating their overall frequency of appearance requires assessing a large number of species in a phylogenetic framework. I suggest to combine phylogenetically explicit information on non-random co-existence patterns (Parra et al. 2010) and phylogenetically explicit information on changes in rates of trait evolution (Eastman et al. 2011). Such an approach would allow (i) to evaluate whether a correlation between co-existence patterns and evolutionary processes exists, and (ii) to ask a whole set of new questions. Does the environmental niche in groups of related species that coexist evolve at slower rates compared to groups that do not co-occur? Do some trait's evolutionary rates not co-vary with coexistence patterns? And are these, consequently, not mediating community assembly? Interesting questions that remain to be answered.

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APPENDIX I VASCULAR PLANTS AS SURROGATES OF BUTTERFLY AND GRASSHOPPER DIVERSITY ON TWO SWISS SUBALPINE SUMMER PASTURES

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In review in "Biodiversity and Conservation".

Summer pastures in the Swiss Alps are currently affected by land-use changes that cause a decrease in biodiversity. Although these habitats make up one third of the whole Swiss agricultural area, direct payments dedicated to support their management are very low. Current political instruments do not support efforts to conserve the biodiversity in these areas, but a vegetation-based approach as the one implemented in the permanently utilized agricultural areas is under discussion. However, available studies evaluating the surrogate value of vascular plants for other (particularly animal) taxa have yielded inconsistent results, and very few have been conducted in habitats at high elevations. We investigated the extent to which vascular plants are adequate surrogates for butterfly and grasshopper diversity, examining the congruence of species richness and community similarity in two heterogeneous subalpine pastures in the Swiss Alps. Results at the species richness level (Spearman's rank correlation) varied widely according to the study site and taxa assessed. In contrast, at the community similarity level (Procrustean randomization tests with Bray-Curtis similarity), congruencies between vascular plant and invertebrate taxa were generally highly significant. We therefore recommend the use of community similarity as a basis for estimating biodiversity patterns. Our results suggest that conservation measures aimed primarily at enhancing the floristic diversity of subalpine grasslands are also likely to benefit butterfly and grasshopper diversity, at least at the local scale.

Introduction

Agricultural activities have shaped Swiss alpine landscapes over centuries, and contributed greatly to their biological diversity (Bätzing 2003). The subalpine grasslands used as summer pastures have especially high nature value, harbouring up to three times as many species as the forest they replaced (Zoller and Bischof 1980). However, changing social and economic conditions have affected land use in alpine regions, with subalpine pastures developing in diverging ways; whereas productive and accessible areas are now managed more intensively, remote, steep and less productive areas are underused or even abandoned. Both trends tend to cause a decrease in biodiversity (MacDonald et al. 2000; Tasser and Tappeiner 2002), and without measures to counteract them, the summer pastures in the forest belt will soon lose much of their conservation value (Mack et al. 2008).

To halt the decline of biodiversity in agricultural landscapes, many European countries including Switzerland have introduced agri-environmental schemes (Kleijn and Sutherland 2003). Since 1999, Swiss farmers have been eligible for direct payments provided they manage their permanently utilized agricultural area according to specified criteria (BLW 1998). One of these is that at least 7% of the agricultural land should be managed as ecological compensation areas (ECAs), which may include extensively managed meadows and pastures, and traditional orchards. Furthermore, an ordinance introduced in 2001 entitles farmers to receive result-oriented financial support for ECAs that meet certain standards of ecological quality (BLW 2001). In this context, ecological quality is defined by the presence in sufficient quantity of certain plant taxa considered to be of conservation value. However, alpine summer pastures are not supported by these instruments because they are not regarded as permanently used agricultural land (BLW 2007). Thus, although the pastures in the Swiss Alps and Jura Mountains make up one third of the total Swiss agricultural area (Baur et al. 2007), they accounted for less than 4% of direct agricultural payments in 2010 (BLW 2011). It is becoming increasingly clear that more financial support will be needed to ensure that these pastures continue to be used for grazing during the summer period (Mack and Flury 2008), and an extension of the national agri-environmental schemes to include the ecological quality of summer pasture areas is currently under discussion. Such a development would help protect the biodiversity of subalpine grasslands while supporting the livelihoods of farmers who use these areas (Klimek et al. 2008; Wittig et al. 2006).

The administration of such a scheme, however, would present practical difficulties because monitoring biodiversity in subalpine grasslands is no simple matter. As the whole biodiversity cannot be recorded (McGeoch 1998; Noss 1990), surrogate indicators are widely used in conservation biology and planning (Caro and O'Doherty 1999; Favreau et al. 2006). Although this approach saves time and money, it depends upon a crucial assumption - that the indicator taxa can serve as a surrogate for a broader range of taxonomic diversity (Howard et al. 1998; Prendergast et al. 1993). Vascular plants are commonly used because they are relatively easy to sample and identify, react sensitively to environmental conditions, and support a large number of animal species (e.g. Marini et al. 2007; Niemelä and Baur 1998). The use of selected vascular plants for assessing ecological quality is the basis of the result-based approach in both Switzerland and Germany (Oppermann and Gujer 2003; Wittig et al. 2006). This approach has been shown to be both efficient and well accepted by farmers (Klimek et al. 2008), though studies investigating the surrogate value of plants in general, and subsets of plant species in particular, for other (primarily animal) taxa have produced results in support (Duelli and Obrist 1998; Nipperess et al. 2012; Panzer and Schwartz 1998; Pharo et al. 1999; Sauberer et al. 2004) and in contradiction (Billeter et al. 2008; Chiarucci et al. 2007; Chiarucci et al. 2005; Niemelä and Baur 1998; Santi et al. 2010; Vessby et al. 2002). Furthermore, very few of these studies have considered the relationships between plant and invertebrate diversity in subalpine areas (Favreau et al. 2006).

The aim of this study was to investigate the adequacy of various measures of plant species richness and composition as surrogates for the diversity of diurnal butterflies and grasshoppers in the summer pasture habitat. We examined the small-scale congruencies of species richness and community similarity patterns in two highly heterogeneous subalpine pastures in the Swiss Alps.

Methods

Study Sites and Sampling Design

The investigation was carried out on two subalpine pastures around the villages of Mesocco (46°23’31’’N/9°13’58’’E) and Guarda (46°46’33’’N/10°08’59’’E) in the Canton of Grisons in southeastern Switzerland (Figure 1). The mean annual precipitation measured at weather stations close to the survey sites between 1961 and 1990 was 1864 and 693mm year-1 for Mesocco and Guarda, respectively (Schweizerische Meteorologische Anstalt 2008). Crystalline underlying rocks dominate at both sites (Reinhard et al. 1962). Both pastures have

a south-west aspect, are approximately 1.5km2 in area, grazed by cattle, and situated in marginal locations just above the present tree line (1762-2064m elevation in Mesocco and 2097-2410m in Guarda). The vegetation is composed of a mosaic of grasses and herbs, mainly Nardus stricta, and dwarf ericaceous shrubs and junipers (Juniperus spp.). Fifty-four plots of 30m x 30m (900m2) were located with a systematic sampling (randomly placed regular grid with a 155m resolution; see Fig. 1) on each pasture. Hazardous regions of the pasture had to be excluded from the survey resulting in a final sample of 49 plots at Mesocco and 50 plots at Guarda.

Plant, Butterfly and Grasshopper Sampling

Data collection was carried out between June and September 2010. To account for the heterogeneity in the plots, plants were surveyed in nine quadrats of 1m2 placed systematically within the 900m2 plot (Figure 1). The plant cover was estimated using the Braun-Blanquet scale (Braun-Blanquet 1964) and the taxonomy follows (Lauber and Wagner 2001). We also recorded the presence of any additional species absent from the nine quadrats by searching the whole plot for 30 minutes. Two plant data sets were derived in this way: a "cover data set" from the nine 1m2 quadrats consisting of abundances acquired from the Braun-Blanquet scale, and a "presence data set" of all species recorded in the 900m2 plot. We recorded all adult butterflies (Rhopalocera, Hesperiidae and Zygaenidae) and grasshoppers (Ensifera and Caelifera) by walking over the 900m2 plot in a serpentine-like transect (see Balmer and Erhardt 2000; Figure 1). Both taxa were identified visually, and grasshoppers also acoustically, during sunny days with little or no wind between 10:00 and 17:00. The butterfly survey was performed on five occasions, and the grasshopper survey on two occasions. Some butterflies (mostly individuals belonging to the Genera Erebia, Pyrgus, Adscita and Zygaena) were retained for accurate genital identification, but all other insects were released where they had been caught. Butterfly nomenclature followed Bühler-Cortesi (2009) and grasshopper nomenclature Baur et al. (2006). For both insect taxa, data of different sampling times were pooled at the plot level prior to data analysis.

Measures of Species Richness and Quality

Four plant measures were used to test congruence of the butterfly and grasshopper data at the species richness level: 1) total number of plant species, hereafter species richness, derived from the "presence data set"; 2) the number of plant target and characteristic species of nature conservation value considered to indicate ecological quality of Swiss agricultural surfaces

(BAFU & BLW 2008), hereafter plant 'quality species'; 3) the average number of species within the nine 1m2 quadrats (α diversity), and 4) the number of species among quadrats (β diversity), derived by subtracting α diversity from the total number of species (γ diversity) in the nine quadrats (Formula 1 in Wagner et al. 2000). For the butterfly and grasshopper taxa two measures were used for comparison: 1) species richness (i.e. total number of species), and 2) the number of 'quality species' (i.e. number of target and characteristic species).

Comparisons between the four plant measures and the two measures for butterflies and grasshoppers were made by means of pairwise Spearman's rank correlation tests as the data were not normally distributed. Because only one grasshopper quality species, Gomphocerus sibiricus, was recorded at Mesocco, comparisons using the number of grasshopper quality species were not performed for this site. Spatial autocorrelation in the data was examined using a Mantel test with 10'000 permutations. Only species data in Guarda were found to be spatially autocorrelated, so for statistical inference partial Spearman's rank correlations between the variables controlling for the effect of geographical variables were determined in all cases (Dale and Fortin 2009). Analyses were performed using the "ppcor" (Kim 2011) and "vegan" (Oksanen et al. 2012) packages of the statistical program R (R Core Team 2012). We did not apply sequential Bonferroni corrections for correlations in agreement with the objections of Moran (2003) and similar studies dealing with surrogate indicators (Fattorini 2010; Mandelik et al. 2012).

Community Similarity

We used procrustean randomization tests (PROTEST; Jackson 1995) for pairwise community similarity comparisons between the plant and insect taxa at each site. This approach was preferred to the commonly used Mantel test because of its greater power in detecting congruence between two community matrices (Gioria et al. 2011; Peres-Neto and Jackson 2001). PROTEST attempts to minimize the sum-of-squared deviations between two data matrices by stretching and rotating the points in one matrix, while still maintaining the relative distance between points within each matrix (Jackson 1995). Both the cover and presence plant data sets were used for comparisons with the insect abundance data. Cover data of plants and abundance data for insects were square root-transformed prior to data analysis to improve normality. Bray-Curtis similarity matrices for the species data were then calculated using the transformed cover/abundance data as well as presence data. Because a Mantel test (10'000 permutations) between species assemblages (Bray-Curtis similarity) and the geographic distance matrix revealed spatial autocorrelation in the community data of both

the Mesocco and Guarda sites, a partial PROTEST following the approach of Peres-Neto & Jackson (2001) was performed. This method permits the degree of association of two matrices to be calculated using PROTEST while fixing the potential effect of geographical variables. The matrices were thus standardized using non-metric multidimensional scaling (NMDS), and information on the stress value was used to define the number of axes to use. Four NMDS axes were retained for both the species and geographical matrices. Two steps were used to perform partial PROTEST (Peres-Neto & Jackson, 2011): First, for each axis retained from species NMDS analysis, multiple regressions with all geographical axes were performed. Then the residuals obtained from these multiple regressions were used to carry out pairwise partial PROTEST analysis. The Procrustes correlations ( ) were derived from the symmetric Procrustes residuals (m), which are the sum-of-square residuals between matrices in their optimal superimposition. Because of too many zero values in the matrix of butterfly and grasshopper quality species, it was not possible to carry out a reasonable NMDS and thus PROTEST analysis. Analyses were performed using the "vegan" package (Oksanen et al. 2012) in R (R Core Team 2012).

Results

In Mesocco 318 plant, 44 butterfly and 8 grasshopper species were recorded and identified. In Guarda 332 plant, 52 butterfly and 14 grasshopper species were recorded. More exhaustive species and abundance data for the three taxa are summarized in Table 1. In general, plant, butterfly and grasshopper species richness and abundance at the two sites were similar.

Measures of species richness and quality

The various measures of plant diversity - species richness, number of 'quality' species, α and β diversity - generally showed moderate to strong positive correlations with both the butterfly total species and quality species richness at both study sites (Table 2; Figure 2). At Mesocco, however, the correlations between the α diversity of plants and both total and quality species richness of butterflies did not quite reach the level for significance ($p=0.064$ and $p=0.113$). At Guarda, the correlations between the four plant measures and species richness of grasshoppers were not significant (Table 2; Figure 3).

Community similarity

Cover as well as presence community data of vascular plants coincided well with insect abundance at both sites. Apart from the insignificant relationships between plant species

cover and grasshopper abundance as well as plant quality species cover and butterfly and grasshopper abundance at Mesocco, all relationships between plant and insect community data showed highly significant positive correlations at both sites (Table 3; Figures 4 & 5).

Discussion

In this study of two subalpine pastures, we found generally strong and positive congruence between various measures of plant diversity (species richness patterns, α diversity and β diversity) and the abundance of butterflies and grasshoppers, though with some differences between the insect taxa and the sites assessed. However, congruencies between plant and invertebrate communities were consistently strong for both taxa and sites regarded.

One explanation for the congruence between plants and butterflies is their producer-consumer relationship. Most butterflies are dependent upon one or a few plant species during their larval stage, while adult butterflies also show more or less strong associations with certain flowering plants for nectar (e.g. Tudor et al. 2004). These relationships are well reflected in our results obtained, which agree with previous studies and confirm that vascular plants can be taken as a good surrogate for butterflies both at the levels of species richness (Panzer and Schwartz 1998; Pearman and Weber 2007) and community similarity (Santi et al. 2010; Su et al. 2004).

The results for grasshoppers are less clear. A significant positive correlation between vascular plants and grasshoppers was found in a study of low altitude agricultural landscapes in Austria (Sauberer et al. 2004). However, our data only partially support their findings, with differences between the two study areas, particularly at the level of species richness. Although grasshoppers also depend upon vascular plants, most species are generalists and their diets may include a relatively wide range of plant species. Thus, aspects such as biomass production, vegetation structure or microclimate are more important in shaping grasshopper distribution than plant species richness per se (Detzel 1998; Szövényi 2002). Furthermore, the number of grasshopper species surveyed at these higher altitudes was relatively low. Results for the species richness and their interpretation should therefore be treated with caution as the small species pool might have affected the statistical outcomes, at least in terms of reduced power to obtain significant results. Despite the few species, the total numbers of grasshoppers at both sites were high, making community similarity a more appropriate measure for comparison. Oertli et al. (2005) also found stronger correlations between grasshoppers and bees using community similarity data instead of species numbers. Nevertheless, merely eight rather generalist grasshopper species at the Mesocco site caused the species assemblages to be

particularly homogeneous among plots with rather different vegetation communities (Fig. 5). With almost twice as many species, the grasshopper community similarities in Guarda showed much higher correspondence with those of vascular plants.

Besides the direct associations of vascular plants with butterflies and grasshoppers, the taxa also share similar responses to environmental factors and management (e.g. Marini et al. 2009). Oertli et al. (2005) suggested that congruence of diversity patterns is more likely at local scales when there is a strong environmental gradient to which the taxa respond similarly. For example, Su et al. (2004) concluded that the congruence of plant, bird and butterfly communities was due to a hydrological gradient. The sites in our study were topographically very heterogeneous with very variable micro-climatic conditions, which are likely to have influenced the distribution of species and thus potentially underlie congruencies.

The spatial scale at which species diversity is studied may also affect the congruence among taxa (Favreau et al. 2006; Wolters et al. 2006); whilst local environmental conditions are likely to be more important at the small scale, evolutionary, historical or climatic factors may be more influential at larger scales (Heino et al. 2009). Although it is reported that taxa show better correspondence in species richness at larger scales (Wolters et al. 2006), the importance of studying congruence among taxa at a small scale has been repeatedly emphasized (Vera et al. 2011; Weaver 1995). It can be argued that this is the scale at which conservation actions and management planning such as agri-environmental schemes are mostly carried out, and therefore the scale at which congruence analyses would be of most practical relevance. Even though ours was a small-scale study, vascular plants performed well as surrogates of butterflies and grasshoppers at the community similarity level. Despite divergent results of surrogate studies, vascular plants have been repeatedly recommended as one of the best indicators for overall biodiversity (Duelli and Obrist 1998; Kati et al. 2004; Sauberer et al. 2004).

In order to capture different aspects of species diversity patterns, we also used measures of plant diversity other than species richness. Whilst Spearman rank correlations between the average plant diversity and invertebrate taxa within samples (α diversity) were lower, correlation values for the diversity among samples (β diversity) were as high as those for the species richness, and in some cases even higher. Not only the number of plant species present but also the spatial turnover of plant species among neighbouring areas is thus an important aspect in shaping the diversity of butterflies and grasshoppers in this subalpine habitat,

possibly reflecting the considerable topographical heterogeneity in the areas studied. This outcome is in contrast to the results of Kessler et al. (2009), who compared species richness and β diversity of plants and animals in Indonesia and found that β diversity of one taxon had very limited indicator potential for species richness of other taxa. The differing results could be explained by the fact that Kessler et al. (2009) included different habitat types, and that some taxa showed divergent preference for specific habitats, while our study only included alpine grasslands. Nevertheless, the community similarity approach, which not only incorporates the abundances of single species but also provides information on the species identity, yielded strong congruencies between plants and invertebrates. This agrees with previous studies on surrogate indicators that found measures based upon community similarity to be more effective than those based species richness (Bilton et al. 2006; Lovell et al. 2007; Su et al. 2004).

As the whole biodiversity cannot be assessed, specific taxa are often used as surrogates for other taxa to reduce the time and cost of monitoring in conservation planning. The effort can be reduced even further if a subset of species from one taxon, rather than recording all species, is used. This approach is already utilized in agri-environment schemes in Switzerland and Germany, where result-oriented payments to the farmers and thus biodiversity conservation of agricultural areas are based on a list of plant indicator species (Oppermann and Gujer 2003). Our results indicate that the plant 'quality species' represent well species richness and community similarity of butterflies and grasshoppers. These findings partly contradict results from Vellend et al. (2008) who found that the strength of correlations for species richness and composition are reduced if only a subset of species is used; on the other hand, they argue that the use of a subset can even maximize statistical power, and that little information is lost by eliminating a small proportion of rare species (e.g. below 10%). In any case, the success of a subset relative to all species will highly depend on how the species are selected; whilst the subsets used by Vellend et al. (2008) were obtained by removing species randomly, the so-called quality species used here were selected specifically for their conservation value.

In summary, our investigation of subalpine Swiss summer pastures has shown strong overall correspondence between vascular plants and two insect taxa, butterflies and grasshoppers, especially when community diversity measures are used rather than simple species richness. We therefore recommend the use of community similarity data in studies evaluating surrogacy, as this measure takes species identity as well as species abundance into account, just as do standard diversity indices. Although our correlations do not reach the value of 0.75

recommended by Lovell et al. (2007) as a criterion for accepting vascular plants as a suitable surrogate for other taxa, given the consistently strong correlations at the community level, we are confident that vascular plants represent an acceptable surrogate for butterflies and grasshoppers (though caution is required when the species pool is small). This in turn implies that conservation measures aimed primarily at protecting the plant diversity of subalpine grasslands will also benefit butterflies and grasshoppers, at least at the local scale at which management decisions are usually made. We expect a similar profit for other invertebrate taxa such as bumblebees or syrphid flies that show strong interactions with plants. Nevertheless, animal groups not directly related to plants are likely to show poor congruence. Therefore, Finch & Löffler (2010) recommended the use of one group of invertebrates in addition to plants as indicator for conservation planning in alpine areas. Given the lack of studies in these areas, more effort should be dedicated to investigate surrogacy, especially for invertebrate groups not directly related to specific plant taxa. Furthermore, we conclude that recording a subset of vascular plants (the 'quality' species) can substantially increase the efficiency of assessments without reducing their predictive value as surrogates for other taxa. Our results suggest that the Swiss 'quality species' are well selected to represent insect species richness and community similarity. They, therefore, are well suited for assessing result-oriented policy.

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Tables

Table 1 Summary statistics for the plant, butterfly and grasshopper taxa collected in 900 m^2 plots at the study sites Mesocco (n=49) and Guarda (n=50). Insect data were pooled over the summer season (five surveys for butterflies and two for grasshoppers). SE = Standard Error.

	Measure	Sampling area[b]	MESOCCO			GUARDA		
			Min-Max	Mean±SE	Total	Min-Max	Mean±SE	Total
VASCULAR PLANTS	Species richness (total number of species)	9x1 m^2 & 30min walk	36 - 110	67.1 ± 2.7	318	41 - 116	76.1 ± 2.3	332
	Number of quality species[a]	9x1 m^2 & 30min walk	5 - 36	19.6 ± 1.2	94	5 - 44	24.6 ± 1.1	100
	Alpha diversity (mean number of species)	9x1 m^2	6 - 23	13.3 ± 0.7	-	12 - 30	21.7 ± 0.6	-
	Beta diversity	9x1 m^2	12 - 56	30.9 ± 1.5	-	18 - 62	37.7 ± 1.5	-
BUTTERFLIES	Species richness	900 m^2 plot	3 - 14	7.8 ± 0.5	44	4 - 22	10.1 ± 0.5	52
	Number of individuals	900 m^2 plot	8 - 76	31.0 ± 2.1	1517	7 - 93	48.6 ± 3.2	2429
	Number of quality species[a]	900 m^2 plot	0 - 4	0.9 ± 0.2	19	0 - 10	4.0 ± 0.3	25
GRASSHOPPERS	Species richness	900 m^2 plot	2 - 6	3.5 ± 0.1	8	2 - 10	5.5 ± 0.2	14
	Number of individuals	900 m^2 plot	1 - 316	65.4 ± 8.7	3207	22 - 329	127.4 ± 9.6	6369
	Number of quality species[a]	900 m^2 plot	0 – 1	-	1	0 - 6	2.7 ± 0.2	7

[a]Number of species indicating ecological quality in Swiss agricultural landscapes (BAFU & BLW 2008).
[b]Vascular plant species were recorded in 9 x 1 m^2 quadrats within the 900 m^2 plots as well as during targeted search for further 30 minutes walk through the plot. To calculate alpha and beta diversity only the 9 x 1 m^2 quadrats were considered. Insects were recorded on a serpentine-like transect covering the whole plot.

Table 2 Pairwise comparisons between four plant measures and two measures for butterflies and grasshoppers in the study sites Mesocco (n=49) and Guarda (n=50). Partial Spearman's rank correlation and significance values (p, in brackets; significant values in bold) are reported.

STUDY AREAS	COMPARISONS	Plant measures			
		Species richness	Quality species	α-diversity	β-diversity
	Sampling area	900 m^2	900 m^2	9 x 1 m^2	9 x 1 m^2
MESOCCO	Butterfly species richness	**0.362 (0.009)**	**0.337 (0.016)**	0.266 (0.064)	**0.396 (0.004)**
	Butterfly quality species	**0.302 (0.034)**	0.273 (0.057)	0.230 (0.113)	**0.353 (0.011)**
	Grasshopper species richness	**0.387 (0.005)**	**0.408 (0.003)**	**0.492 (<0.001)**	**0.375 (0.007)**
	Grasshopper quality species[a]	-	-	-	-
GUARDA	Butterfly species richness	**0.579 (<0.001)**	**0.668 (<0.001)**	**0.551 (<0.001)**	**0.550 (<0.001)**
	Butterfly quality species	**0.426 (0.001)**	**0.472 (<0.001)**	**0.460 (<0.001)**	**0.404 (0.003)**
	Grasshopper species richness	0.245 (0.087)	0.164 (0.259)	0.139 (0.343)	0.270 (0.057)
	Grasshopper quality species	0.227 (0.114)	0.148 (0.309)	0.052 (0.722)	0.224 (0.120)

[a] This comparison was not made because of the presence of only one quality grasshopper species at the Mesocco site.

Figures

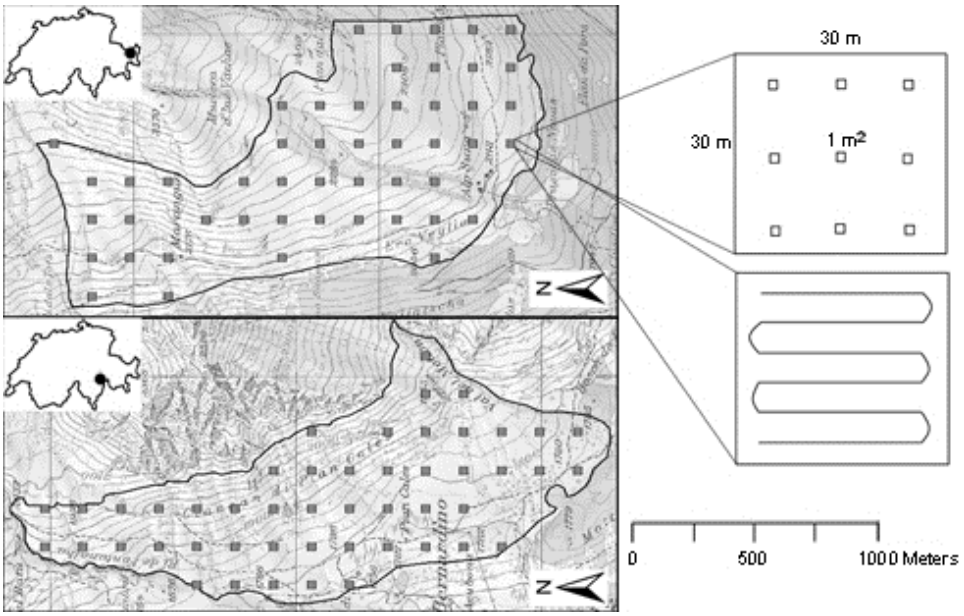

Fig. 1 On the left, the pasture areas in Guarda (top) and Mesocco (bottom) showing the boundary and the systematically located plots. The Topographic Map 1:25,000 (2012 ©Swisstopo) is shown in the background. On the right, the sampling design used for plants (top) and insects (bottom)

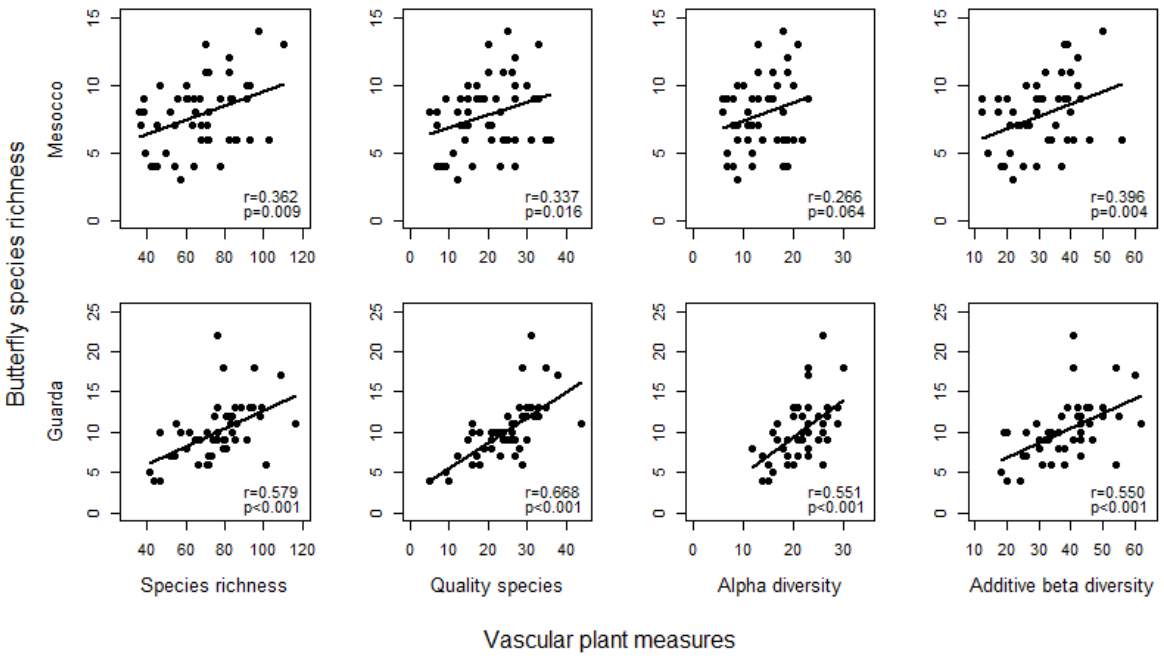

Fig. 2 Four plant measures against butterfly species richness in Mesocco (n=49) and Guarda (n=50). Spearman's rank correlations (r) and significances (p) are reported in the single graphs. Regression lines are shown to simplify interpretation.

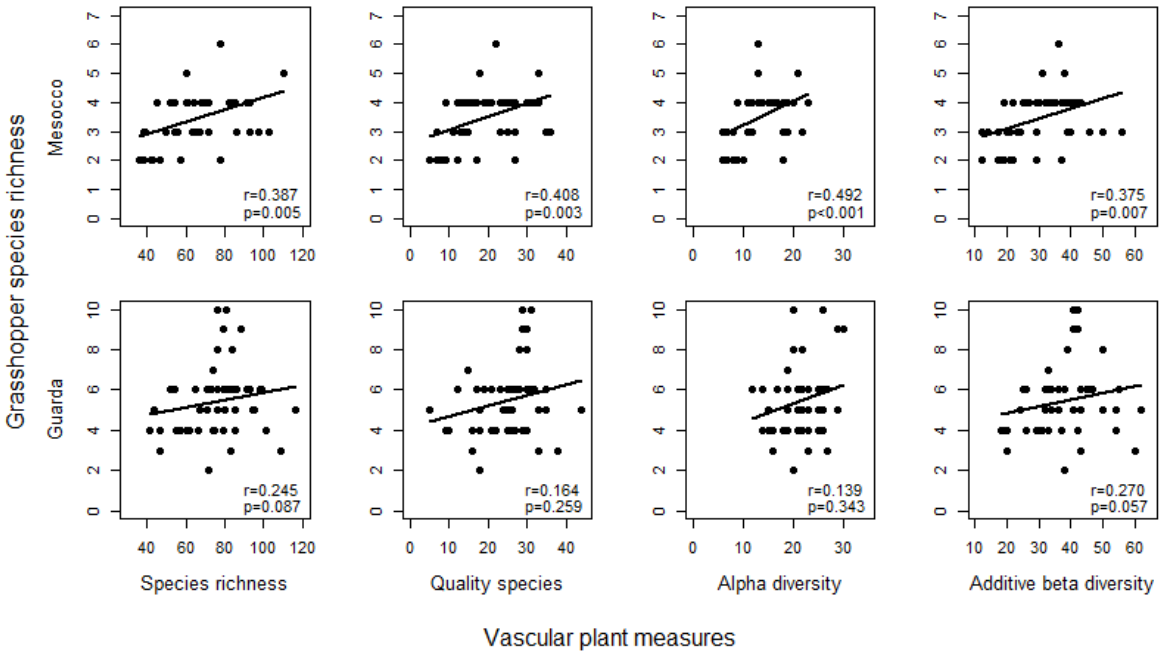

Fig. 3 Four plant measures against grasshopper species richness in Mesocco (n=49) and Guarda (n=50). Spearman's rank correlations (r) and significances (p) are reported in the single graphs. Regression lines are shown to simplify interpretation.

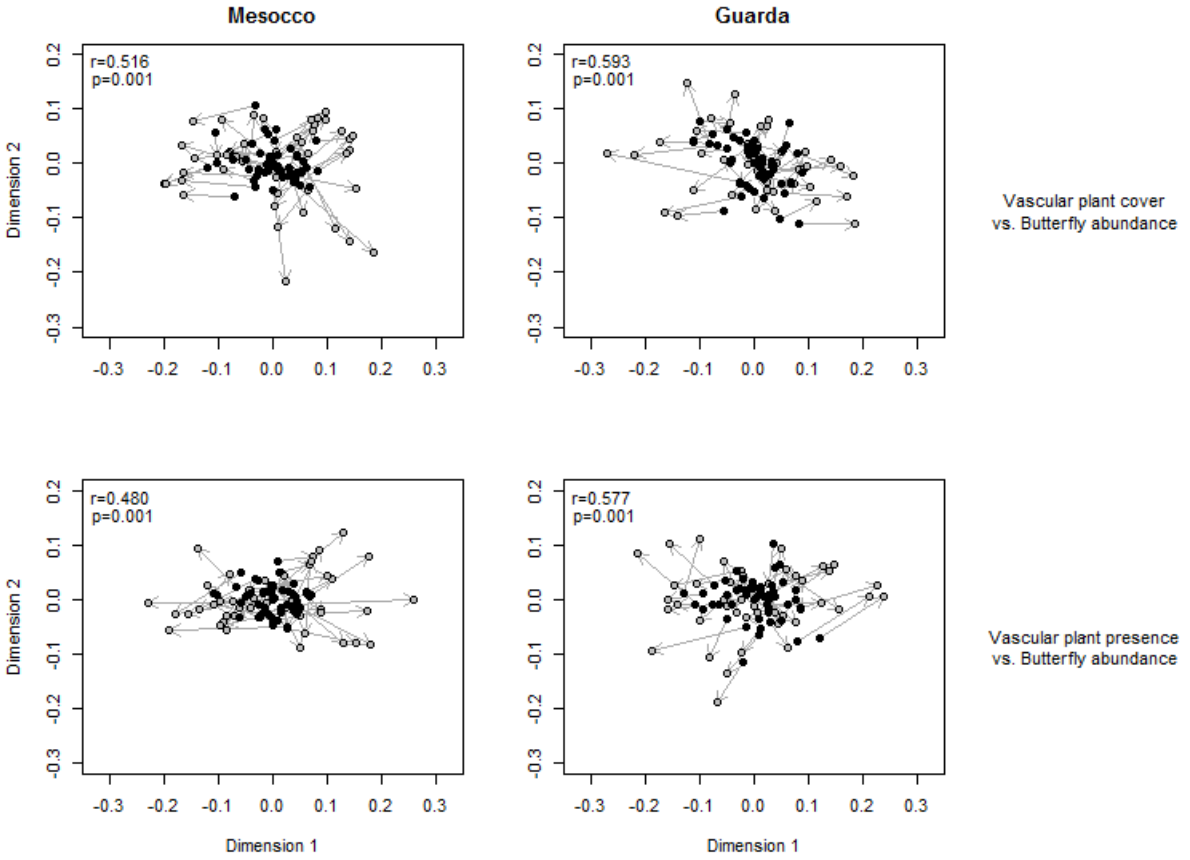

Fig. 4 Procrustean superimposition plots between vascular plant cover or presence and butterfly abundance in Mesocco (n=49) and Guarda (n=50). Procrustes correlations (r) and significances (p) are reported in the graphs. The procrustean errors are shown in the graphs as arrows, the length of which illustrates the amount of difference between the community similarities (Bray-Curtis similarity) of the vascular plant and butterfly taxa. The arrows start from the original rotated data (butterflies, black circles) and points to the target data (vascular plants, grey circles).

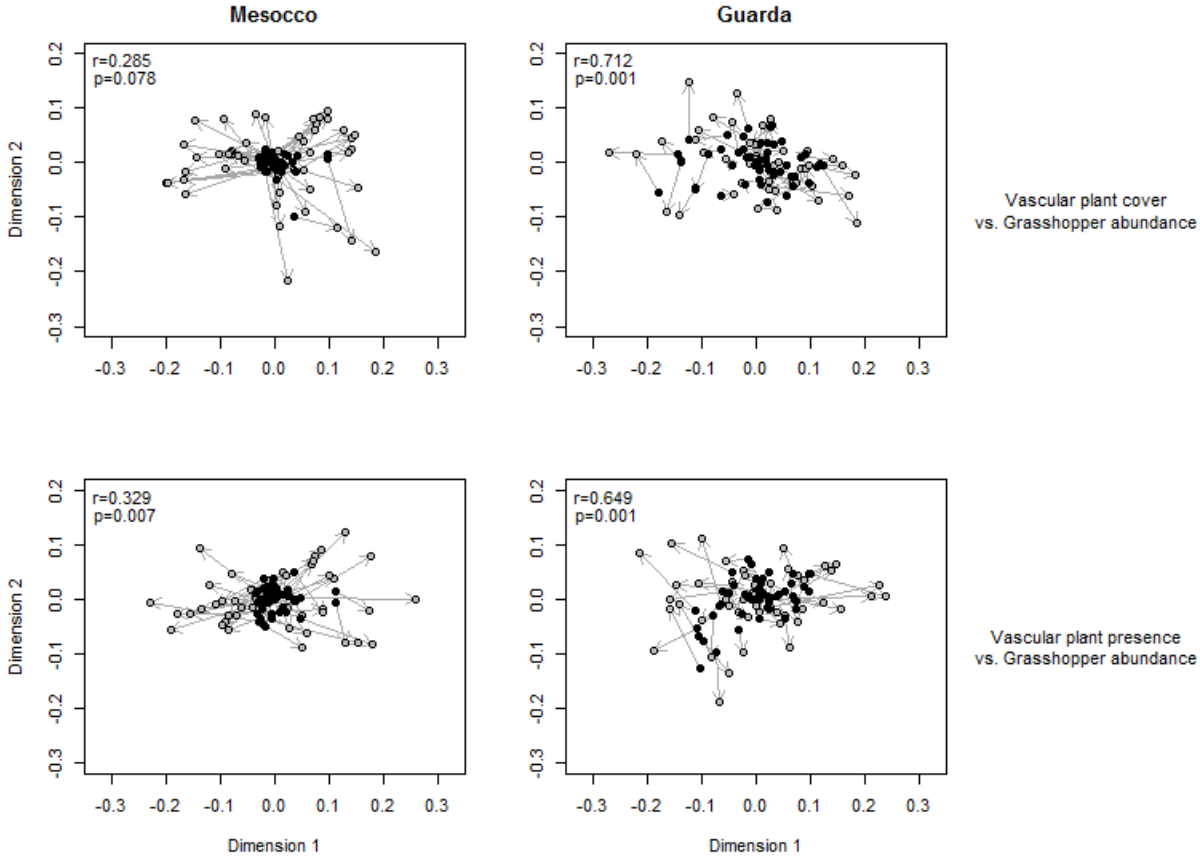

Fig. 5 Procrustean superimposition plots between vascular plant cover or presence and grasshopper abundance in Mesocco (n=49) and Guarda (n=50). Procrustes correlations (r) and significances (p) are reported in the graphs. The procrustean errors are shown in the graphs as arrows, the length of which illustrates the amount of difference between the community similarities (Bray-Curtis similarity) of the vascular plant and grasshopper taxa. The arrows start from the original rotated data (grasshoppers, black circles) and points to the target data (vascular plants, grey circles). It is worth to note the homogeneous grasshopper communities in Mesocco compared to Guarda

APPENDIX II ASSESSING RAPID EVOLUTION IN A CHANGING ENVIRONMENT

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Climate change poses a serious threat to species persistence. Effective modeling of evolutionary responses to rapid climate change is therefore essential. In this review we examine recent advances in phylogenetic comparative methods, techniques normally used to study adaptation over long periods, which allow them to be applied to the study of adaptation over shorter time scales. This increased applicability is largely due to the emergence of more flexible models of character evolution and the parallel development of molecular technologies that can be used to assess adaptive variation at loci scattered across the genome. The merging of phylogenetic and population genetic approaches to the study of adaptation has significant potential to advance our understanding of rapid responses to environmental change.

Opinion

Assessing rapid evolution in a changing environment

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Climate change poses a serious threat to species persistence. Effective modelling of evolutionary responses to rapid climate change is therefore essential. In this review we examine recent advances in phylogenetic comparative methods, techniques normally used to study adaptation over long periods, which allow them to be applied to the study of adaptation over shorter time scales. This increased applicability is largely due to the emergence of more flexible models of character evolution and the parallel development of molecular technologies that can be used to assess adaptive variation at loci scattered across the genome. The merging of phylogenetic and population genetic approaches to the study of adaptation has significant potential to advance our understanding of rapid responses to environmental change.

Evolutionary response to climate change

The speed and scope of ongoing anthropogenic climate change are potentially unmatched in Earth's history [1] and present biodiversity in general, and single species in particular, are likely to be faced with extraordinary circumstances [2]. The resulting ecological disruption and modification will likely be irreversible, with the most dramatic consequence being the extinction of species [3,4]. It is therefore essential to assess potential species responses. These responses can extend from range-limit alterations, which allow tracking of suitable habitat and thereby the promotion of species persistence at a global scale, to tolerance towards new conditions through either phenotypic plasticity or adaptation, which help species avoid local extinction. There is increasing evidence of species declining in abundance [3,5], which potentially underscores the constrained dispersal abilities of many organisms threatened by changing climate, altered land use and increasing anthropogenic habitat fragmentation [6]. These patterns highlight the importance of understanding the potential for species to adapt to climate change *in situ*.

Species characteristics, such as life history traits, as well as the magnitude and degree of exposure to environmental change will affect the relative importance of dispersal and adaptive mechanisms in species reaction to climate change [7–9]. Adaptation to rapidly changing environment has attracted considerable attention from, amongst others, those studying quantitative genetics [10], microevolutionary processes acting on phenology [11,12], and candidate loci that may confer adaptation [13]. These and other studies [14,15] make use of experimental approaches that determine the mechanisms of adaptation and the loci involved. This combined effort has led to some understanding of the limitations on adaptive response to climate change.

Identifying the capabilities of species for adaptation also has wider applications across ecological research and conservation, for example by changing how ecological niche models are built. In particular, species lacking the potential for rapid adaptation will require newly developed hybrid models that combine phenomenological and process-driven

Glossary

Adaptive landscape: a hypothetical response surface that expresses the relative fitness of individuals or populations as a non-linear function of genotype or genotype frequencies, respectively.
Brownian motion process: a random progression of character traits in which the character value for each species changes randomly in direction and magnitude in a temporally uncorrelated fashion.
Epistatic effects: these occur when the effect on phenotype by an allele at a locus is contingent upon the alleles present at a second locus.
Population genomics: the study of the genomes (in whole or part) of organisms from several populations within a single species that is becoming feasible due to new high-throughput sequencing techniques.
Mode of selection: this is characterised by the effect of selection in relation to the distribution of phenotypes that exists before selection occurs. Selection is directional when values on one side of the mean exceed, on average, values on the opposing side.
Ornstein–Uhlenbeck process: a stochastic evolutionary process that generalises Brownian motion by introducing an optimum value for the trait under consideration. Depending on selection strength, the trait is held near the optimum value such that the pull toward the optimum value is stronger as the trait drifts away.
Phenotypic plasticity: variation in a value of an observable character that occurs as an effect of environment and may or may not confer adaptation.
Phenotypic space: multivariate space of sufficient dimensions to accommodate the values of multiple traits measured on multiple individuals.
Phylogenetic constraint: the propensity of particular lineages to resist adaptation to a specific optimum.
Phylogenetic signal: the positive correlation between the degree of genetic relatedness of species and their phenotypic similarity.
Pleiotropy: the positive or negative correlation between two aspects of an individual's phenotype that results from the aspects both being influenced by one or more genes.
Tempo of evolution: the acceleration and deceleration of evolutionary rates and the description of the conditions for rates to be rapid or slow.

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elements that allow for both demographic and migratory responses [16]. Species that have the potential for rapid adaptation will also require a new generation of models. These will need to capture adaptive responses of populations that were in a suitable habitat before the environmental change and so are responding to a shift in favourable conditions as well as those populations which were originally in or have recently migrated to previously unsuitable sites [17]. Until now, theory was not sufficiently advanced to allow the combination of ecological niche concepts and ideas of species evolution into a unified approach to the study of adaptation in large number of species at once. Here, we address emerging attempts to combine phylogenetic and population genetic approaches to the study of evolutionary patterns and rates of adaptation to rapid environmental changes, emphasising the increasing flexibility of the phylogenetic framework.

Adaptive response: Scaling up from one species to many

The merging of population genetics theory with phylogenetics to study evolving ecological patterns could introduce an exciting new scientific approach for investigating how evolution shapes biodiversity. However, this can only be achieved by examining comprehensively large numbers of species at once. Rapid adaptation over a few generations is probably common in a wide range of taxa [11,14,15,18,19], and the phenomenon has been extensively studied using population genetics approaches. In particular, populations at range margins have been a productive research focus [20] because marginal populations are likely to be disproportionately more exposed to ongoing global changes compared to populations from areas central to a species' range [21]. In general, adaptation is a common and widespread microevolutionary phenomenon that is essential in the long term as a response to environmental change [22]. The current challenge is to understand how the potential for these responses is distributed among populations and, ultimately, among species.

One approach to scaling up the study of adaptive responses from specific populations to a whole set of species is based on the variation in rates and tempo of phenotypic evolution between species or clades. Evolutionary rates tend to vary with the strength and mode of selection and the capacity for adaptive divergence of populations due to their intrinsic demographic and genetic characteristics [23]. Under rapid change of the environment away from optimal fitness values of individuals, as expected under future climatic scenarios, a population will rapidly decline in size as maladaptive alleles are removed by selection [20]. This decline in population size is dependent on the amount of genetic variation available in populations [24] and the demographic process must support the spread and increased frequency of adaptive alleles for the population not to go extinct [25,26]. Following this rapid initial response to selection and accompanying population decline, the rate of adaptation slows as average fitness within the population approaches its maximum value and/or additive genetic variance is exhausted. Further, theoretical models predict that conditions that (i) flattened selective gradients, (ii) reduced gene flow towards local populations (that is, isolate populations from maladaptive alleles) and (iii) increased population size or growth, all enhance potential rates of adaptation [20,26]. These conditions are likely to vary between species and analysis of phenotypic evolution within populations of a broad range of species suggests that these predictions hold across divergent time scales and phylogenetic relationships [27].

However, genetic mechanisms that act to promote trait evolution and adaptation over long evolutionary times are probably different from those generating genetic variation and adaptation to changing environment at the intraspecific level. In particular, epistasis and negative pleiotropic effects reduce the probability that mutations become established in sequences coding for proteins [28]. In particular, a relatively small number of genes or even regions of genes accumulate mutations more easily than the average coding site. This can create phenotypic variation and allow rapid divergence of ecologically-significant traits that favour species divergence and radiation [29–31]. It is very probable that particular lineages have relatively higher capacity to harbour consequent adaptive changes and can therefore

Box 1. Evolution of C_4 photosynthesis

An example of the effect of global climatic changes on adaptation can be found in the grass family (Poaceae). This plant group, known for its economic and ecological importance, is host to one of the most striking examples of convergent genotypic evolution [62], which was triggered by an adaptive response to decreasing atmospheric CO_2 concentration [32]. The C_4 photosynthetic pathway is a suite of biochemical and anatomical adaptations that enhances photosynthetic performance in plants under high-temperature and low-CO_2 environments when compared to the standard C_3 pathway [63]. This suite of adaptations has appeared independently over 18 times in the diversification of the grass family [62,64]. Analyses of several genes that are responsible for key elements in the pathway [62,65,66] show that recurrent episodes of adaptive evolution at the genetic level occurred during each independent origin of the pathway. This evolutionary independence stands in contrast to the observed phenotypic convergence found in all C_4 plants [63].

The evolution of the C_4 pathway in grass lineages occurred at different times, with the first appearance approximately 30 My ago. The appearance of the C_4 pathway in multiple clades within the grass family provides an opportunity for a statistical application of comparative methods. Christin *et al.* [32] incorporated the change in CO_2 concentration through time into a Markov model of character evolution. Rates of transition between C_3 and C_4 states, estimated by maximum likelihood, were allowed to be different before and after a time threshold defined as the CO_2 concentration that physiologically advantages C_4 plants. The impact of this climatic change on the rate of appearance of the C_4 character was then tested by comparing, using a likelihood ratio test, a null model forcing the rates to be identical before and after the threshold with the alternative model that did not restrict the rates. This study showed that the appearance of the C_4 pathway in grasses is associated with an abrupt decrease in atmospheric CO_2 levels in the Oligocene [32]. The accumulation of numerous biochemical and morphological modifications required for a functioning C_4 metabolism suggests that some lineages are predisposed to acquire these changes [67] in response to changing environmental conditions. This raises the question of whether such biochemical flexibility is generally associated with the potential for plants to adapt to changing environments. Other characteristics or traits may be regularly associated with adaptive potential (Box 2).

leave a trace of 'residual evolvability' in their descendant species (Box 1).

These arguments suggest that lineage specific characteristics must be considered if we want to jointly assess the potential for adaption over a broad set of species. The advantage of such an approach is the gain in statistical power achieved by analysing several independent events of adaptation at once (e.g. [32]). The approach we advocate here consists of studying historical and potential evolutionary rates in substantial numbers of species simultaneously and is embodied in a new phylogenetic approach to discover rapid evolution. Recent research demonstrates the utility of comparative methods for studying rates of trait evolution, for example during adaptive radiations and other diversifications [33–36]. These efforts are supported by an ever-widening taxonomic breadth, and rapidly expanding technology for high-throughput DNA sequencing, which will soon allow the routine reconstruction of highly resolved phylogenies of several hundreds of species. Large numbers of sequences from multiple populations and species suggests the feasibility of a better and deeper integration of population processes into phylogenetic comparative methods. Such a combined comparative approach might thus serve as a framework to bridge intra- and interspecific levels, which we see as an essential endeavour if we are to better understand the adaptive potential of species.

Evolutionary models in comparative analysis

With most comparative methods it is not possible to model the effect of directional or stabilising selection toward an optimal (adaptive) character state (Figure 1a) because these methods are based on the underlying assumption that Brownian Motion (BM) processes closely approximate character evolution [37]. The BM process is akin to genetic drift and can be used as a good null model, but is not an effective model for the processes of adaptive evolution. Furthermore, many current comparative studies compound this problem by only testing for presence or absence of a phylogenetic signal, without properly testing any suitable evolutionary model (whether adaptive or not). This is probably due to a common misconception that phylogenetic signal and evolutionary rate are necessarily related. Traits that confer adaptation can vary greatly in the levels of phylogenetic signal they display and, presumably, in their levels of resistance to further adaptation [38,39]. Simulations have shown that measures of phylogenetic signal cannot be used to distinguish different evolutionary processes because many evolutionary scenarios can generate similar levels of phylogenetic signal [40]. Measures of phylogenetic signal are thus not suited to test adaptive response between species [31].

To account for adaptation, modifications of the assumption of BM in comparative methods have centred on either weakening the strength of BM to the point that the analysis becomes non-phylogenetic [41] or transforming the branch lengths of a phylogenetic tree in an attempt to match the assumptions of the BM model [42]. The difficulty in the first approach is that BM is a pure drift process, and one does not obtain a model of selection from a random BM process by merely weakening it. Although the second approach of transforming phylogenetic trees results in statistically valid analyses, distortion of the phylogeny confuses interpretation and makes it difficult to infer the validity of the hypothesised evolutionary process and to estimate the rate and tempo of trait evolution. The listed limitations inherent in many current implementations of comparative methods suggest that other, more flexible approaches are required to identify and compare the adaptive potential of species (Box 2).

In response to the fundamental limitation of BM-based methods, tools have been proposed that model selection directly. Following the suggestion of Felsenstein [43], Hansen [44] proposed to model adaptive evolution by means of the Ornstein–Uhlenbeck (OU) process. The model [45] was initially developed to assess how far a population mean phenotype could deviate from a selective optimum, given the strength of selection and the size of the population, but extension of this method has allowed the modelling of adaptation to be extended over larger evolutionary time scales [43,44,46,47]. While this extension renders the model incompatible with the presence of phylogenetic constraint for the trait under consideration [44], the development of

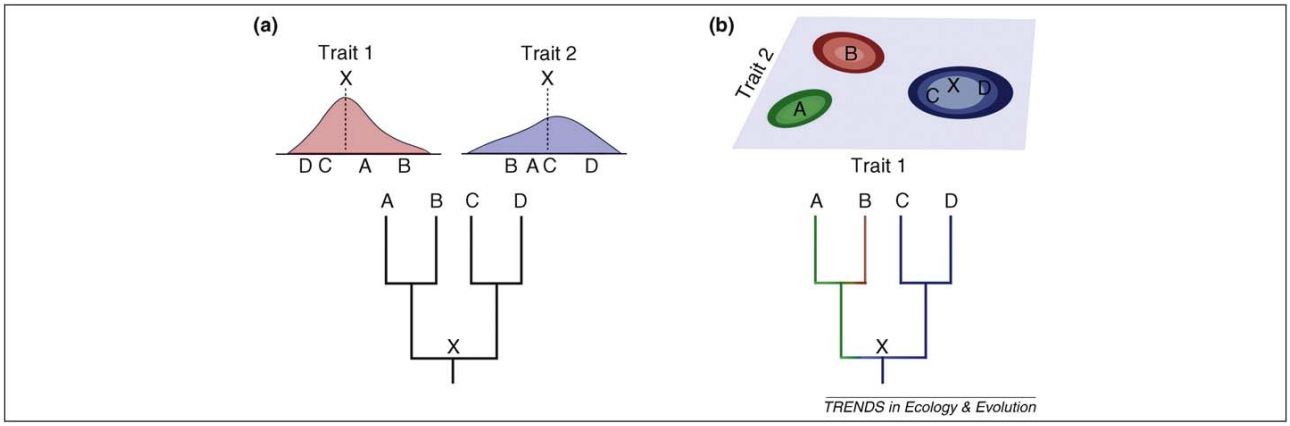

Figure 1. Shift in the study of trait evolution, here for two ecological traits and four species, A–D. The current approach to studying trait evolution **(a)** uses a single trait value per species and each trait is considered independently. A Brownian null model describes trait evolution from the most common ancestor (X), with the mean trait value of each species being the same as that of X. An alternate approach **(b)** is to model ecological traits as multivariate distributions in order to incorporate population-based measures of trait values (i.e. polymorphism within species), covariation (non-independence) between traits, and allow for different evolutionarily optimal trait values among lineages (e.g. for A and B).

Box 2. Comparative methods and niche evolution in a plant and a lizard

The factors influencing rates and distribution of phenotypic evolution have been a longstanding question. A recent study by Smith and Beaulieu [68] investigated how growth form and climate tolerance correlate in five angiosperm groups. The climate tolerances of species were estimated from data on species distributions. The mean values for 19 WorldClim [69] variables served as proxy tolerance estimates for each species. This approach assumed an adaptive component to among-species differences in these values. Phylogenetic multivariate analysis of variance assessed how among-species differences varied between species that were grouped by growth form (woody or herbaceous). The observed differences were compared to those expected based on a Brownian model of evolution.

In particular, Smith and Beaulieu presented a flexible model that allowed rates of accumulation of evolutionary changes in climate tolerance to differ between woody and herbaceous species. The authors compared evolution of climate tolerance in this model to evolution in an alternative null model that forced an identical rate of adaptation in both growth forms. The accumulation of difference in climate tolerance depended on plant growth form. Woody lineages exhibited slower phenotypic and molecular evolution, which could constrain how woody species respond when faced with environmental changes. The result suggests that adaptation to future climate change will depend on the life-history characteristics of species. The implication is that other variability in life history may also be associated with the rate of adaptation to climate change.

In a comparable example, the effect of life-history on adaptation to the temperature variability among habitats was investigated by Labra *et al.* recently in *Liolaemus* lizards of South America [70]. These authors modified the approach of Hansen *et al.* [47] so that trait optima followed changes in habitat temperature. Labra *et al.* then tested whether physiological performances were similar between habitats. The results indicated that adaptation to thermal conditions evolved rapidly with little phylogenetic constraint, potentially indicating rapid adaptive shifts as lineages became specialised for specific habitats. In contrast, other characteristics, such as body size and critical thermal minimum did not indicate adaptation to thermal environment. However, permissible body temperature could also be constrained, resulting in congeneric species having similar habitat preferences. This points to the problem of interpreting trait variation that is strongly structured among lineages and the degree that this structure represents constraints on evolution. In summary, traits such as habitat preference, growth form and others can be evaluated across related clades to determine the association of particular character values with the extent of adaptation to both spatial and temporal environmental trends.

OU process models is a key step linking microevolutionary processes to macroevolutionary patterns though the incorporation of parameters that describe an adaptive landscape [48]. Indeed, this model has also been used to study trait evolution on population level phylogenies [49].

Further extensions of the OU process have allowed the optimum value of a phenotype to change through time using a BM model [47]. Maximum likelihood approaches have been developed to estimate evolutionary optima in different lineages [46] and to allow the optimum itself to change through time [47]. This reconciles tests of adaptive evolution that combine optimal trait values with phylogenetic constraint. If not considered, such constraints could lead to rejection of the hypothesis of adaptation simply because of spurious phylogenetic effects [31].

The actual BM and OU processes used in these studies constrain the rate of character evolution to be constant through time along the studied lineages (although in some formulations, OU models can lead to observed exponential divergence between lineages). The stochastic processes expressed in these models are, therefore, inadequate for modelling lineages which change between periods of stasis or rapid change in character values during lineage evolution [27]. This limitation will introduce biases that could, because of unmet model assumptions, result in a wrong rejection of the null hypothesis of neutral (i.e. BM) character evolution. There is ample room for more complex models of phenotypic character evolution and the time is ripe for these further developments. Changes in rates of evolution along a lineage have already been used in phylogenetics [32,50], and the development of Markov chain Monte Carlo techniques should lead to a refinement of available evolutionary models. Other types of stochastic processes, for example those based on compound stochastic processes or heterogeneous Markov chains, could permit the movement of trait values between adaptive optima that are scattered in phenotypic space [43]. Because they allow for rate changes through time and differences between lineages, the more rugged evolutionary courses of character values modelled by these stochastic processes could be more appropriate for modelling adaptive response to environmental changes.

Population level comparative methods

Scaling up from one species to many in studies of adaptive potential is important and the new evolutionary models that are emerging will play a key role. However, the information available in population level data, represented by polymorphism in the trait values measured, should not be excluded from such analyses. On the contrary, population-level data will provide additional information needed to assess the importance of the characteristics affecting species specific rates of adaptation. However, current implementations of comparative methods assume that traits means are measured without error for each species. In practice, the mean phenotype is usually estimated from small samples, with the assumption that intraspecific variation is negligible [44]. This assumption can lead to small sample artifacts and inflation of type I error rates [51,52]. These effects are especially important when comparing closely related species for which the amount of interspecific variance is small compared to intraspecific variation, and between there is a good deal of shared evolutionary history [53]. Accounting for intraspecific variation in comparative methods remains a significant challenge. However, meeting this challenge is essential for the future development of comparative methods. These developments are sure to be enhanced by next generation sequencing technologies as these will bring the necessary population genomic data necessary to use the developments foreseen.

There have been a number of attempts to use population-level data within comparative methods. The first involves partitioning the variance of character evolution into phylogenetic (i.e. interspecific) and error components [54]. The trait distribution within species can then be

modelled by adding nonphylogenetic variation around the species means, assuming a nonphylogenetic burst of adaptation [55]. This burst can represent adaptive differences among populations, such as local adaptation due to climate variability and introduces an additional form of evolution independent of certain phylogenetic constraints. The effects of intraspecific variation can be modelled, following the work of Lynch [56], by allowing each character to have variation within species and for the measurements of different characters within species to be correlated (Figure 1b) [52]. These developments focus mainly on continuous characters, which is an appropriate approach for describing the climate tolerances of species or populations, but polymorphism in discrete traits at the species level can also be incorporated using Wright's [57] threshold model [58]. An appealing property of this model is that it allows for covariation among multiple characters (Figure 1b), which is difficult to accomplish with other types of models for discrete characters. Recent developments also suggest most comparative methods could benefit from research equating phylogenetic relationships with inbreeding coefficients between species [59]. Although interesting, it is not yet clear if this new approach will be useful for modelling rapid adaptation of natural populations to climate change.

Finally, accounting for within-species phenotypic variation within phylogenetic comparative analysis of evolutionary rates can be achieved by allowing multiple character values per species (Figure 1b). A straightforward strategy is to simply gather the population-level values into an empirical distribution for each species for the trait of interest. The comparative analysis is then repeated, using Monte Carlo techniques, by sampling from this empirical distribution [35,60]. This results in confidence intervals estimates for the parameter of interest, for example the rate of adaptation between species or the force of selection in a OU process, but does not explicitly account for intraspecific variation. A statistically more complex, but biologically more appropriate way, to account for within-species variation is to explicitly represent multiple populations per species and calculate both within- and between-species contrasts [53]. With this approach, intraspecific variation is first estimated for each species independently, assuming that individuals share all their evolutionary history. The contrasts between individuals within a species and between species are independent, because they are weighted differences in trait values. The novel and interesting aspect is that the contrasts between different traits have a covariance, which represents the phenotypic covariance of the original traits. The estimated trait covariation can, alongside genetic experiments, give an estimation of the selective covariances of traits [61]. Although this estimation is difficult [53], it is made possible by the use of Markov chain Monte Carlo techniques, whether in a full maximum likelihood setting using importance sampling or in a Bayesian framework. The general and widespread use of these approaches open a new avenue for bringing comparative methods into the era of population genomics. The additional information coming from population-level data will be very useful to model the tempo of adaptive rates within each species, which in turn could prove useful to examine differences in rates of adaptation between species more accurately.

Future directions and conclusions

Overall, the theoretical and statistical developments we have presented in this paper have the potential to move studies that use comparative methods into a new direction, by providing models that fit real biological data better than previous models. This is being accomplished by incorporating multiple correlated traits and intraspecific variability into comparative methods (Figure 1) and will take advantage of recent statistical and computational advances to estimate adaptive trait optima and phenotypic covariance [53]. This is particularly significant for studies of the evolving environmental niche, which is in essence a multidimensional construct with several correlated parameters (e.g. tolerance ranges of potential evapotranspiration and temperature). The recent developments of comparative methods open the possibility for a new set of approaches that will result from the fusion of phylogenetics and population genetics. This will allow biologists to test hypotheses about rates of adaptation by integrating data on ecological conditions, individual phenotypes and genotypes, and their frequencies in populations. This challenging endeavour is essential for a better understanding of the evolution of genes, phenotypes, populations and communities under rapidly changing environmental conditions. New approaches that combine population-based information with phylogenetic comparative methods could improve our understanding of the potential for rapid evolution and should help to identify lineages that are 'frozen' evolutionarily and which could be important foci for conservation efforts.

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APPENDIX III MUTUALISM WITH SEA ANEMONES TRIGGERED THE ADAPTIVE RADIATION OF CLOWNFISHES

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Background: *Adaptive radiation is the process by which a single ancestral species diversifies into many descendants adapted to exploit a wide range of habitats. The appearance of ecological opportunities, or the colonization or adaptation to novel ecological resources, has been documented to promote adaptive radiation in many classic examples. Mutualistic interactions allow species to access resources untapped by competitors, but evidence shows that the effect of mutualism on species diversification can greatly vary among mutualistic systems. Here, we test whether the development of obligate mutualism with sea anemones allowed the clownfishes to radiate adaptively across the Indian and western Pacific oceans reef habitats.*

Results: *We show that clownfishes morphological characters are linked with ecological niches associated with the sea anemones. This pattern is consistent with the ecological speciation hypothesis. Furthermore, the clownfishes show an increase in the rate of species diversification as well as rate of morphological evolution compared to their closest relatives without anemone mutualistic associations.*

Conclusions: *The effect of mutualism on species diversification has only been studied in a limited number of groups. We present a case of adaptive radiation where mutualistic interaction is the likely key innovation, providing new insights into the mechanisms involved in the buildup of biodiversity. Due to a lack of barriers to dispersal, ecological speciation is rare in marine environments.*

Particular life-history characteristics of clownfishes likely reinforced reproductive isolation between populations, allowing rapid species diversification.

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Mutualism with sea anemones triggered the adaptive radiation of clownfishes

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Abstract

Background: Adaptive radiation is the process by which a single ancestral species diversifies into many descendants adapted to exploit a wide range of habitats. The appearance of ecological opportunities, or the colonisation or adaptation to novel ecological resources, has been documented to promote adaptive radiation in many classic examples. Mutualistic interactions allow species to access resources untapped by competitors, but evidence shows that the effect of mutualism on species diversification can greatly vary among mutualistic systems. Here, we test whether the development of obligate mutualism with sea anemones allowed the clownfishes to radiate adaptively across the Indian and western Pacific oceans reef habitats.

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Conclusions: The effect of mutualism on species diversification has only been studied in a limited number of groups. We present a case of adaptive radiation where mutualistic interaction is the likely key innovation, providing new insights into the mechanisms involved in the buildup of biodiversity. Due to a lack of barriers to dispersal, ecological speciation is rare in marine environments. Particular life-history characteristics of clownfishes likely reinforced reproductive isolation between populations, allowing rapid species diversification.

Keywords: Ecological speciation, Diversification, Comparative method, Evolutionary rate, Brownian Motion, Pomacentridae

Background

The concept of adaptive radiation has been central to evolutionary biology since Darwin's work on Galapagos finches [1-3]. The general understanding of this process is that rates of ecomorphological changes and species diversification will be increased by ecological opportunities offering available resources untapped by competing species [4]. Ecological opportunity can arise for four main reasons [5], the most widely described being the colonisation of geographically isolated areas with depauperate fauna (e.g. cichlid fishes in East-African Great Lakes [6]). The process is similar in the aftermath of a mass extinction event, which allows surviving species to radiate rapidly by filling the available empty niches [7]. Modification of a resource can also trigger native species radiation as demonstrated by the radiation of *Lupinus* in high-elevation habitats that appeared during the Andean uplift [8]. Finally, the appearance of a trait allowing new interactions with the environment, or key innovation, can create an opportunity for species radiation [9]. For example, the evolution of antifreeze glycoproteins found in notothenioid fishes of Antarctica is thought to have triggered their adaptive radiation by allowing survival in extreme environments [10]. In an analogous manner to key innovations, the evolution of mutualistic interactions can provide access to previously inaccessible resources. For instance, phytophagous insects host mutualistic microbes, which enable

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the breakdown and digestion of plant compounds by the insects [11]. While a plethora of case studies showing adaptive radiation driven by ecological opportunity offered by one of the aforementioned possibilities exist [9], examples involving mutualism are scarce (but see [12]). Since hosts shifts have allowed ecological speciation in a wide range of organisms, including coral-dwelling fish [13,14], there is a possibility for ecological speciation to occur in mutualistic systems. However, results from empirical and theoretic studies give contradictory evidence on the effect of mutualism on species diversification [15,16]. The topic is thus still debated and in need of further case studies.

The clownfishes (or anemonefishes; subfamily Amphiprioninae) are a group of 30 species within the damselfish family (Teleostei; Perciformes; Pomacentridae; [17]) and are emblematic species of coral reefs (Figure 1A & 1C). Their distribution spans from the Indian to the western Pacific Oceans (Figure 1B) with their highest species richness found in the Indo-Malay archipelago where up to nine species have been observed in sympatry [18]. Their complex association with sea anemones is now a textbook example for mutualistic interactions [19-21]. Clownfishes are left unharmed by the otherwise lethal nematocysts of the sea anemone tentacles. This ability is thought to come from a protective mucous coat that prevents the discharge of the nematocysts [22] and allows clownfishes to settle in sea anemones. The protection against predators provided by the sea anemones is a direct advantage for clownfishes. Likewise, clownfishes chase the predators of the sea anemones. Furthermore, waste ammonia excreted by the clownfishes is used by the endosymbiotic dinoflagellates living in the sea anemone tissues, which makes it a three-way interaction [19,21]. The efficiency of the protection provided by the sea anemone is demonstrated by the extraordinary life span of clownfishes (ca. 30 years recorded for *Amphiprion percula*), which is twice as long as any other damselfish and six times greater than the expected longevity for a fish of that size [23].

While species of clownfishes can develop mutualistic interactions with up to ten species of sea anemones (Table 1), a large variation in host usage exists within the clade [20]. Eight host sea anemones have a widespread distribution and two (*Heteractis malu* and *Macrodactyla doreensis*) have more restricted ranges, but are distributed around the centre of diversity for the clownfishes, making interaction between most clownfishes and host species geographically possible [20]. Although geographically widespread, sea anemone species differ in their preferred habitat (e.g. reef zonation, substrate, depth; [24]). It was shown that coexistence of multiple clownfish species was possible because of difference in host and habitat utilisation [18]. It is therefore possible that the appearance of mutualism was the key innovation that allowed the clownfishes to diversify in ecological niches associated with the different sea anemones species. However, this hypothesis has never been tested thoroughly.

Examples of ecological speciation events are rare in marine ecosystems. This is likely due to the fact that

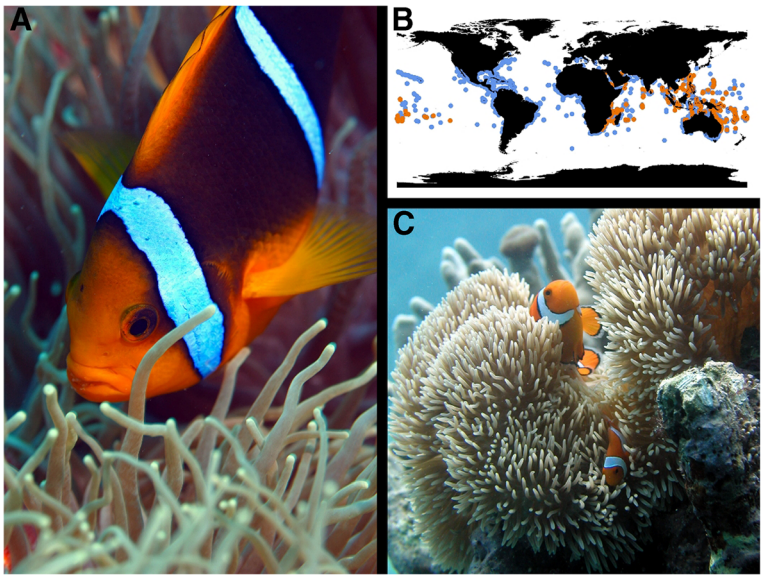

Figure 1 ***Clownfishes and sea anemones mutualism, and geographic distribution.*** Illustration of the mutualistic relationship between *Amphiprion chrysopterus* and *Heteractis crispa* **(A)**. The distribution of the damselfishes in blue and of the clownfishes in orange is shown in panel **B**. As for every clownfish species, the female *Amphiprion percula* (on top of the picture of panel **C**, here with *Stichodactyla gigantea*) is bigger than the male beneath.

Table 1 Interaction matrix between clownfishes and their sea anemone hosts

	Cryptodendrum adhaesivum	*Entacmaea quadricolor*	*Heteractis aurora*	*Heteractis. crispa*	*Heteractis magnifica*	*Macrodactyla doreensis*	*Stichodactyla gigantea*	*Stichodactyla haddoni*	*Stichodactyla mertensii*	*Heteractis malu*
Amphiprion akallopisos					+				+	
Amphiprion akindynos		+	+	+	+			+	+	
Amphiprion allardi		+	+						+	
Amphiprion barberi		+		+						
Amphiprion bicinctus		+	+	+	+		+		+	
Amphiprion chagosensis		(+)								
Amphiprion chrysogaster			+		+			+	+	
Amphiprion chrysopterus		+	+	+	+	+		+	+	
Amphiprion clarkii	+	+	+	+	+	+	+	+	+	+
Amphiprion ephippium		+		+						
Amphiprion frenatus		+								
Amphiprion fuscocaudatus									+	
Amphiprion latezonatus				+						
Amphiprion latifasciatus									+	
Amphiprion leucokranos				+	+				+	
Amphiprion mccullochi		+								
Amphiprion melanopus		+		+	+					
Amphiprion nigripes					+					
Amphiprion ocellaris					+		+		+	
Amphiprion omanensis		+		+				+		
Amphiprion pacificus					+					
Amphiprion percula				+	+		+			
Amphiprion perideraion				+	+	+	+			
Amphiprion polymnus				+		+		+		
Amphiprion rubrocinctus		+					+			
Amphiprion sandaracinos				+					+	
Amphiprion sebae								+		
Amphiprion thiellei				(+)					(+)	
Amphiprion tricinctus		+	+	+					+	
Premnas biaculeatus		+								

Known interactions are shown by plus signs. Field records are lacking for *A. chagonsensis* and *A. thiellei*, the most probable host is shown between parentheses. The species status of *A. leucokranos* and *A. thiellei* is debated as they may be natural hybrids [20].

long-distance dispersal is common among marine organism [25]. However, clownfishes are known to have high larval retention to natal reefs [26,27]. They are also known to produce species-specific calls [28,29] that differ among geographic populations [30]. Such properties are likely to have reinforced reproductive isolation by reducing gene-flow between clownfishes populations and thus facilitated ecological speciation processes in clownfishes.

In this study, we test whether the evolution of the mutualism with sea anemone in the clownfishes lineage is a key innovation that led to ecological adaptive radiation (sensu [4]). We ensure that the clownfishes are monophyletic by building a phylogeny for the Pomacentridae family. Next, we show the occurrence of rapid speciation in the clownfishes by testing whether their diversification rate is higher than that of the other damselfishes. We then use an ordination method on the mutualistic interactions to describe potential ecological niches associated with the sea anemones. We further apply phylogenetic comparative methods to test the association between morphological traits and the putative ecological niches. We finally measure the rate of evolution of the morphological traits to see if they fit the theoretical expectation of faster morphological evolutionary rate [4,31].

Methods

Mutualism and clownfishes phenotype

We collected data on the distribution of clownfishes among the 10 possible sea anemone host species (Table 1; [19,20,32]). We applied a multiple correspondence analysis (MCA) on the matrix of mutualistic interactions between sea anemones and clownfishes. The MCA analysis is the counterpart of principal component analysis for categorical data, which shows the underlying structure present in the dataset. We used the axes of the MCA that explained most of the variance in the subsequent analysis. This allowed us to determine in a multivariate space, the characteristics of the ecological niches used by the clownfishes and provided by mutualistic interactions.

We extracted morphological measurements of the damselfish from the literature (mainly from [33], other sources are listed in the Additional file 1). It is thought that adaptation to sea anemones required modifications of the general shape as well as a change in swimming ability in host specialised clownfish. Indeed, continuous and fast swimming is not needed anymore because specialised species never venture far from their host [34]. We thus collected traits in the literature for all Pomacentridae species present in our phylogeny that are linked with body shape and swimming abilities as well as trophic niche, which is generally linked with habitat in Pomacentridae [35]. This analysis resulted in a matrix of eight morphological traits (maximum standard length, the ratio between standard length and the greatest body depth or "body ratio", the count of hard and soft dorsal-fin rays, the count of soft anal-fin rays, the count of pectoral-fin rays, the number of gill rakers present on the first gill arch and the number of scales which possess a sensory tube or "lateral-line scales"). Standard length and body ratio describe the overall fish shape, which has been shown to be linked with adaptation towards habitats with differing water velocity regimes [36,37]. Fin morphology directly influences fish locomotory ability [38] and gill rakers are used as a proxy for the differentiation along the pelagic-benthic trophic resource axis [39]. The number of lateral-line scales is one of the more pronounced morphological differences between the clownfishes and other damselfishes [40], and may be of importance in the ecological adaptive radiation. It was not possible to take into account intraspecific variation in our analysis and we recorded a single value per trait estimated as the mean of the values obtained from the literature. To diminish potential allometric effects, all traits were log transformed before further analysis.

Phylogeny and divergence time estimation

We assembled DNA sequence data for 196 Pomacentridae species (170/356 damselfishes, 26/30 clownfishes) spanning all genera in the family (Accession numbers available in Additional file 2). Three cichlid species (*Aequidens rivulatus*, *Thorichthys meeki*, *Tomocichla sieboldii*) were included as outgroups [41,42]. The concatenated sequence matrix was 6945 bp long and composed of six mitochondrial and three nuclear gene regions (12S, 16S, ATP6-8, COI, cytochrome b, ND3, BMP-4, RAG1 & RAG2). Each DNA region was aligned separately with MUSCLE [43] and ambiguously aligned nucleotides were removed using Gblocks [44].

After visually checking the alignment, we used BEAST [45] to simultaneously infer the phylogeny and estimate divergence times. We used a relaxed clock model, drawing substitution rates from a lognormal distribution. We partitioned the alignment by gene as it outperformed an unpartitioned analysis in Bayes factors in a similar dataset [41]. We selected, using Akaike information criterion values (AIC), the substitution model that fits best each partition with the function "phymltest" available in the Ape package [46] in R [47] (see the model choice in Additional file 3). We used the only fossil calibration point available for the basal node of the Pomacentridae to obtain absolute divergence time estimates. The fossil that is the earliest record of Pomacentridae (Monte Bolca, Italy) dates back to 50 million years (MY) [48], which we used as minimum age with a lognormal prior (mean = 2; sd = 1.2; prior 5-95% = 51.03-103.2) following [41]. We selected a lognormal prior to allow the basal node of the Pomacentridae to

reach back to ~105 MY, which is the probable age of the Perciformes [49,50]. We performed two parallel BEAST runs, each $5*10^7$ generations long and sampled posterior distributions every 1,000 generations. We checked the convergence of the two chains, optimal sampling of model parameters and estimated the burn-in length in Tracer [45]. After the removal of 10,000 trees as burn-in, we merged both runs and inferred a maximum credibility phylogeny using TreeAnnotator [45]. Finally, we resampled from the posterior distribution 100 trees to be used in further analysis. These time calibrated trees are hereafter referred to as the distribution of chronograms. As BEAST also outputs phylograms having branch lengths given in expected number of substitution per site, we applied the same resampling procedure to get a random sample of 100 phylograms. This allowed us to test our hypothesis on two sets of phylogenies instead of possibly biasing our results by choosing arbitrarily a specific branch length unit [51].

Diversification rate

We used the package Diversitree [52] in R to test whether mutualism with sea anemones is linked with an increased diversification rate in the clownfishes as would be expected under the key innovation hypothesis. We applied the BiSSE method [53], which evaluates jointly the evolution of a binary character (here presence or absence of mutualism with sea anemones), speciation and extinction rates. As we do not have a complete sampling of the Pomacentridae, we used an extension of the method that deals with incompletely sampled phylogenies [52]. A one-rate birth-death model is fitted to the whole tree and compared, using AIC and Likelihood ratio test, with an alternative model allowing two separate rates of speciation and extinction for clownfishes and damselfishes species. In this particular case, the clownfishes are a monophyletic group nested within the Pomacentridae phylogeny. No known clownfishes species has lost the mutualistic behaviour and we therefore forced the loss of mutualism in the model (parameter q10) to a fixed null value. We optimised the other parameters of the model (rates of speciation, extinction and probability of character change) by Maximum Likelihood estimation independently on each of the 100 randomly sampled chronograms to account for phylogenetic uncertainty. The rate of diversification was calculated by subtracting the extinction rate from the speciation rate.

Phylogenetic signal and phenotype-environment correlation

We estimated the phylogenetic signal in the morphological data on each of the 100 phylograms and chronograms with the K [54] and λ [55] indexes as implemented in the Phytools package [56] in R. Assessing the phylogenetic signal of a trait on both phylograms and chronograms can help choose which branch length unit will be the most appropriate for comparative analysis [51]. For both indexes, a value close to 0 is diagnostic of a weak or nonexistent phylogenetic structure, while values close to one are expected if the data follows a Brownian motion (BM) model of character evolution. We performed randomisation tests for the K and a likelihood ratio test for λ to test for an observed phylogenetic signal significantly greater than 0. We repeated the analysis by taking into account only the clownfishes and this time also measuring the phylogenetic signal of the four first axes of the host usage MCA.

Past competition creating character displacement between related species will result in phenotypes that are correlated with resource usage [4]. We assessed whether the morphological traits collected are linked to host usage in the clownfish by measuring the correlation between each of the first four axes of the MCA and the eight morphological traits. We used phylogenetic generalised least squares (pGLS) as implemented in the caper package in R [57]. The λ parameter, which models the phylogenetic dependency of species trait values [55] was estimated by Maximum Likelihood and the model was replicated over each tree present in the samples of phylograms and chronograms. We assessed if the morphological variables explained a significant part of the variance in the model by running an ANOVA on the pGLS output.

Morphological evolutionary rate

We measured the differences in rate of morphological evolution between clownfishes and damselfishes by comparing the fit of a single rate BM model to that of a multiple rate model. It has been shown that other models could better fit the data than BM especially in adaptive radiations [58,59]. We choose to use BM because our goal is solely to compare the relative rate of evolution between groups and not the actual trait values. The single rate model assumes that all lineages accumulate the same amount of morphological variance per unit of time while the multiple model allows clownfishes to have a different rate of evolution than the damselfishes. Both models were specified in the Phytools package [56] that implements the non-censored version of a typical BROWNIE analysis [60]. The best fitting model was selected according to sample size corrected AIC (AICc). We analysed each of the recorded morphological traits on the two sets of 100 trees randomly sampled from the posterior distributions of phylograms and chronograms.

Results

Phylogenetic inference and divergence time

Our maximum credibility phylogenetic tree shows strong support for the monophyly of the clownfishes with a high

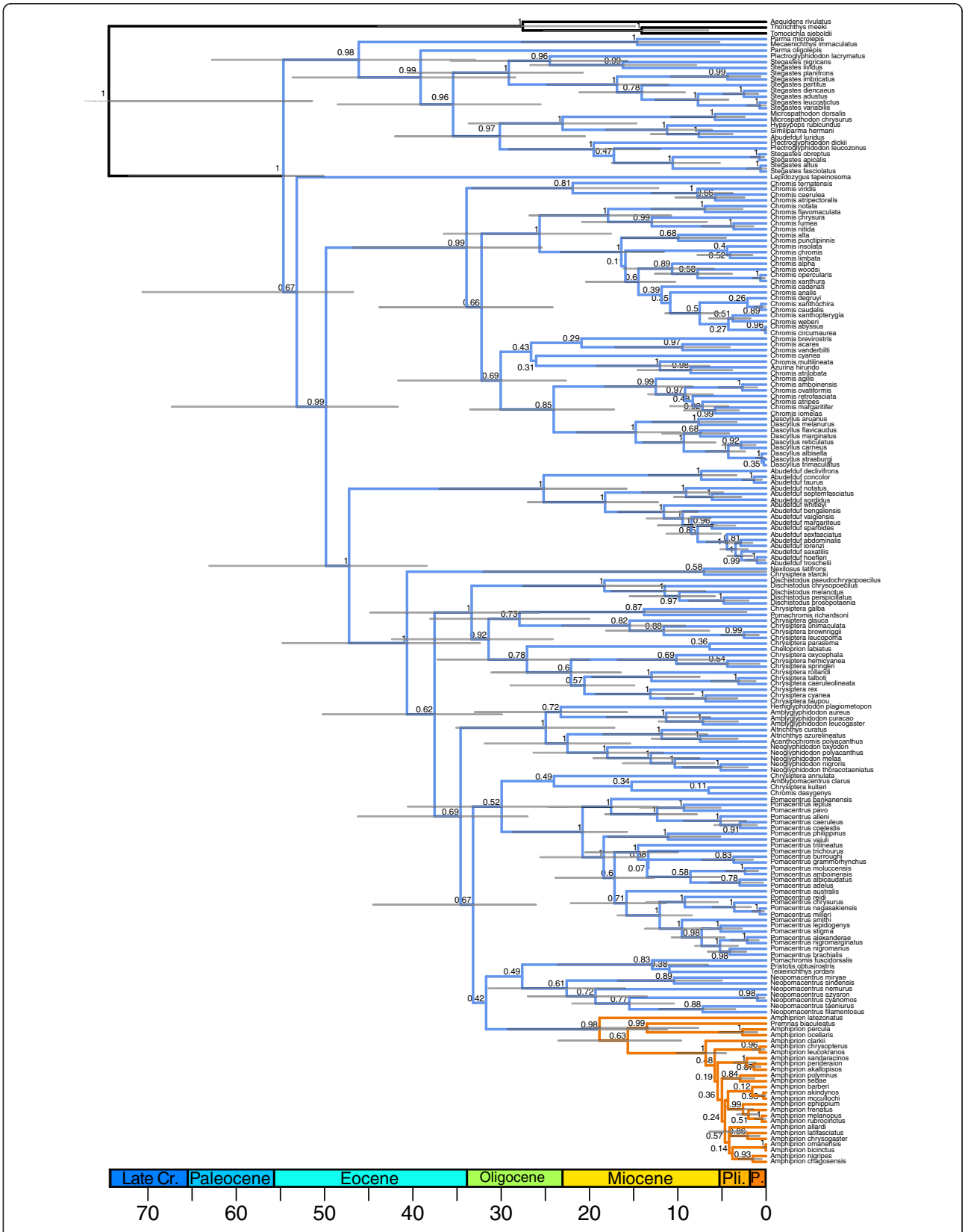

Figure 2 ***Pomacentridae maximum credibility chronogram.*** Outgroup taxa are shown in black, damselfishes in blue and clownfishes in orange. Error bars on node show the dating confidence intervals, scale is in MY. Numbers above nodes indicate Bayesian posterior probabilities.

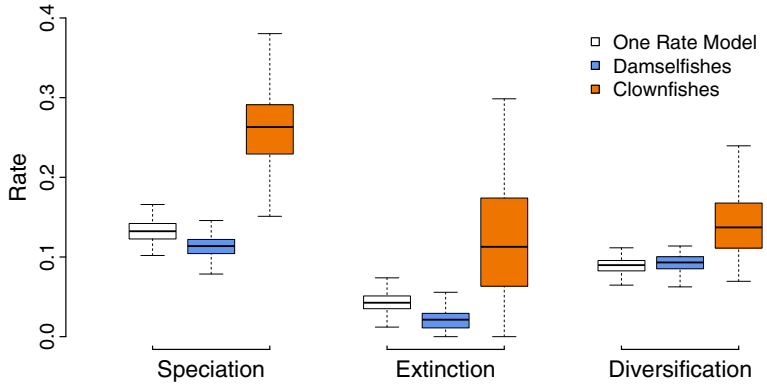

Figure 3 ***Diversification analysis.*** Rates of speciation, extinction and diversification measured on the sample of 100 chronograms. Rates of damselfishes (mean diversification rate = 0.09) are shown in blue and clownfishes (mean diversification rate = 0.14) in orange.

on chronograms. In this case, only the standard length, body ratio, soft anal fin-rays and gill rakers had a faster evolutionary rate in the clownfishes. To verify that the elevated rates found in the clownfishes were not due to the relatively short branches of the clownfish clade we simulated a continuous trait under a BM model with a single rate on our phylogenies. The rates we recovered were not different between clownfishes and damselfishes.

Discussion

We found that clownfishes exhibit patterns that are likely diagnostic of an ecological adaptive radiation via ecological speciation [4]. Following the acquisition of specific ability to interact and live with sea anemones, clownfishes diversified into multiple ecological niches linked with both host (Figure 7) and habitat use. Morphological evolution accelerated and distinct clownfish species developed convergent phenotypes correlated to the host-associated ecological niches.

Pomacentridae phylogeny and diversification

Our inferred phylogeny was congruent with previous work [17,41] and showed with high posterior probabilities that clownfish are monophyletic within the Pomacentridae family (Figure 2). We used the BiSSE method [53] to test if the appearance of mutualism acted as a key innovation and if it is associated with an increase in speciation rate. The BiSSE method is a powerful way to detect shifts in diversification rate linked with a binary trait, but the change of state of the binary trait does not usually correspond to a single monophyletic group as in our case. Nevertheless, we chose the BiSSE method as it takes into account the uncertainty in dating the appearance of mutualism

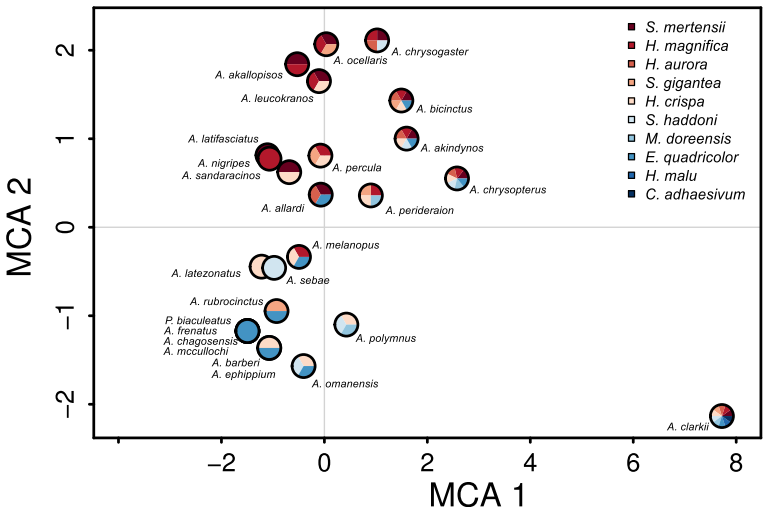

Figure 4 ***MCA of mutualistic interactions (axes 1 and 2).*** Each pie represent a clownfish species and the filling colours correspond to the interacting sea anemone species (see legend in figure). Abbreviations: *Amphiprion*: A, *Premnas*: P, *Stichodactyla*: S, *Entacmaea*: E, *Macrodactyla*: M, *Heteractis*: H, *Cryptodendrum*: C.

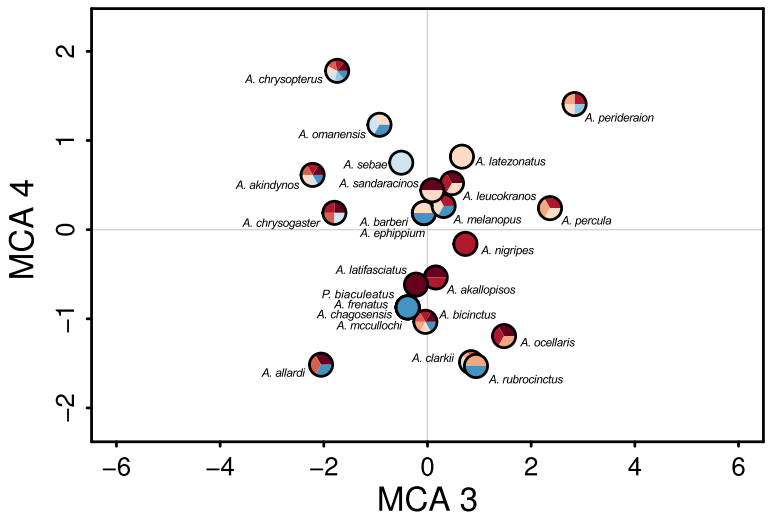

Figure 5 ***MCA of mutualistic interactions (axes 3 and 4).*** Legend as in Figure 4.

on the phylogeny. Indeed, mutualism with sea anemones likely appeared in the common ancestor of the clownfish, but the stem branch of the group is ~13MY long (Figure 2). It is therefore important, when estimating speciation and extinction rates, to take into account the uncertainty in the time estimates that correspond to the appearance of this behaviour. We also constrained the rate of mutualism loss to be null in the BiSSE model. This takes into account the fact that all clownfish are nowadays mutualistic, but it may slightly bias our analysis as it is not impossible that a clownfish species will eventually revert to a non-mutualistic state. However, we do not think that this parameter will influence significantly our results. Other methods exist to infer speciation rates on phylogenies that do not need prior hypotheses on the location of the shift in rate [64]. In a recent paper [41], the likely nodes of diversification rate shifts were inferred for four coral reef fish families using relative cladogenesis test [65] and MEDUSA [64]. The study included the Pomacentridae family and they consistently found across methods that the clownfishes experienced a significant rate increase. The rate shift

Table 2 Phylogenetic signal in the damselfishes

	Phylograms		Chronograms	
	K	λ	K	λ
Standard length	**0.407±0.11**	**0.934±0.09**	0.358±0.10	0.883±0.04
Body ratio	0.205±0.08	**0.954±0.07**	**0.233±0.09**	0.921±0.02
Dorsal fin soft rays	**0.467±0.14**	**0.925±0.06**	0.461±0.14	0.886±0.02
Dorsal fin hard rays	0.513±0.10	**0.914±0.07**	**0.578±0.12**	0.859±0.02
Anal fin soft rays	0.161±0.07	**0.911±0.07**	**0.186±0.08**	0.872±0.03
Lateral line scales	0.856±0.30	**1.026±0.06**	**0.973±0.33**	0.962±0.02
Pectoral fin rays	**0.349±0.12**	0.842±0.06	0.329±0.12	0.842±0.04
Gill rakers	0.237±0.09	**1.011±0.07**	**0.294±0.12**	0.949±0.02

Blomberg's K and Pagel's λ statistics of phylogenetic signal and standard deviation for the damselfish morphological dataset in the posterior distributions of phylograms and chronograms. For each trait, the statistic closest to one is indicated in bold.

Table 3 Phylogenetic signal of morphology and MCA axes

	Phylograms		Chronograms	
	K	λ	K	λ
Standard length	0.186±0.06	0.578±0.07	0.186±0.08	0.647±0.06
Body ratio	0.149±0.09	0.868±0.16	0.147±0.10	0.829±0.08 *
Dorsal fin hard rays	0.201±0.04	0.131±0.24	0.179±0.05	0.085±0.20
Dorsal fin soft rays	0.169±0.07	0.175±0.30	0.150±0.07	0.141±0.26
Anal fin soft rays	0.11±0.09	0.941±0.14 **	0.109±0.10	0.863±0.03 **
Lateral line scales	0.245±0.10	0.686±0.14	0.216±0.09	0.666±0.21
Pectoral fin rays	0.245±0.13 *	0.977±0.15 **	0.239±0.14	0.89±0.08 **
Gill rakers	0.068±0.05	0.721±0.11 *	0.068±0.06	0.738±0.05 **
MCA 1	0.097±0.06	0.322±0.41	0.088±0.05	0.326±0.40
MCA 2	0.033±0.03	0±0	0.032±0.03	0±0
MCA 3	0.108±0.05	0.172±0.12	0.106±0.05	0.285±0.12
MCA 4	0.04±0.03	0.021±0.15	0.038±0.03	0±0

Blomberg's K and Pagel's λ statistics of phylogenetic signal for the clownfish morphological dataset and host usage MCA in the posterior distributions of phylograms and chronograms. Phylogenetic signal significantly different than 0 is signified by asterisk (* = P-values <0.05, ** = P-values <0.01). Significance was assessed with a randomisation test for the K and likelihood ratio test for λ.

Table 4 Correlation between morphological traits and MCA axes

		Phylograms		Chronograms	
	Morphological trait	Coefficients	Error	Coefficients	Error
MCA 1	Standard length	**0.264 ±0.321**	**1.785 ±0.115**	**−0.093 ±0.755**	**2.188 ±0.408**
	Body ratio	0.039 ±1.765	4.480 ±0.468	−0.036 ±1.435	5.618 ±1.423
	Dorsal fin hard rays	**8.617 ±1.941**	**11.096 ±0.715**	3.661 ±4.792	11.074 ±2.168
	Dorsal fin soft rays	**−6.904 ±2.517**	**12.666 ±0.820**	**−9.241 ±3.278**	**12.270 ±1.496**
	Anal fin soft ray	**0.155 ±2.183**	**9.593 ±0.811**	**−2.716 ±4.671**	**11.252 ±3.342**
	Pectoral rays	**−7.830 ±3.415**	**6.951 ±1.247**	−4.235 ±5.400	8.226 ±2.055
	Lateral-line scales	−4.353 ±1.593	6.168 ±0.349	−1.190 ±4.424	7.030 ±-1.230
	Gill rakers	**16.496 ±1.708**	**5.591 ±0.443**	**15.797 ±2.008**	**7.761 ±2.955**
MCA 2	Standard length	−0.920 ±0.085	1.452 ±0.013	−1.012 ±0.057	1.473 ±0.031
	Body ratio	3.505 ±0.143	3.950 ±0.054	3.781 ±0.459	4.029 ±0.024
	Dorsal fin hard rays	3.751 ±0.518	7.569 ±0.115	2.811 ±0.488	7.484 ±0.325
	Dorsal fin soft rays	10.376 ±0.431	7.626 ±0.095	9.263 ±0.831	7.554 ±0.511
	Anal fin soft ray	**−16.490 ±0.543**	**8.321 ±0.074**	**−16.398 ±0.890**	**8.433 ±0.060**
	Pectoral rays	7.134±0.241	5.685 ±0.060	7.356 ±0.747	5.742 ±0.003
	Lateral-line scales	0.024±0.295	4.724 ±0.080	0.253 ±1.082	4.663 ±0.078
	Gill rakers	4.083±0.344	6.23 ±-0.080	4.429 ±1.397	6.274 ±0.189
MCA 3	Standard length	**−2.421 ±0.097**	**1.233 ±0.017**	**−2.439 ±0.179**	**1.227 ±0.013**
	Body ratio	1.134 ±0.157	3.356 ±0.072	1.024 ±0.206	3.355 ±0.010
	Dorsal fin hard rays	−1.219 ±0.472	6.430 ±0.134	−1.458 ±0.415	6.231 ±0.194
	Dorsal fin soft rays	−1.975 ±0.435	6.479 ±0.131	−2.173 ±0.112	6.288 ±0.336
	Anal fin soft ray	−1.51 ±-0.480	7.069 ±0.126	−1.429 ±1.638	7.023 ±0.014
	Pectoral rays	−5.235 ±0.191	4.829 ±0.090	−5.158 ±0.877	4.782 ±0.043
	Lateral-line scales	−0.05 ±-0.300	4.013 ±0.091	0.088 ±1.333	3.883 ±0.026
	Gill rakers	−1.585 ±0.452	5.293 ±0.093	−1.566 ±0.030	5.227 ±0.189
MCA 4	Standard length	1.498 ±0.035	1.247 ±0.015	1.56 ±-0.060	1.248 ±0.010
	Body ratio	**9.968 ±0.191**	**3.393 ±0.032**	**9.783 ±0.670**	**3.413 ±0.018**
	Dorsal fin hard rays	7.273 ±0.567	6.502 ±0.097	7.820 ±0.112	6.337 ±0.180
	Dorsal fin soft rays	0.651 ±0.609	6.551 ±0.073	1.395 ±0.400	6.395 ±0.321
	Anal fin soft ray	9.599 ±0.288	7.148 ±0.065	9.327 ±0.908	7.142 ±0.030
	Pectoral rays	−2.244 ±0.161	4.883 ±0.044	−2.427 ±1.107	4.864 ±0.054
	Lateral-line scales	**−10.471 ±0.154**	**4.058 ±0.083**	**−10.593 ±0.200**	**3.949 ±0.018**
	Gill rakers	−0.620 ±0.151	5.352 ±0.067	−0.742 ±1.517	5.316 ±0.199

The table shows results and standard deviation of pGLS. Results in bold indicate variables explaining significant variation in the dependent variable as shown by the ANOVA on the pGLS output. Median adjusted R^2 of the models on phylograms, MCA 1 = 0.75, MCA 2 = 0.15, MCA 3 = 0.33, MCA 4 = 0.23, and on chronograms, MCA 1 = 0.65, MCA 2 = 0.16, MCA 3 = 0.36, MCA 4 = 0.22.

was either placed at the origin of the clownfish or at the base of the *Amphiprion* crown group. The fact that a method which does not need *a priori* information on the location of the diversification rate shift recovered similar result as in our study confirms the strength of the diversification rate shift that occurred in the clownfish clade.

It should be noted that the extinction rate also increases in the clownfishes (Figure 3). A possible explanation is that during the diversification process, some clownfish lineages did not leave any descendants as they were ecologically replaced by other more competitive clownfish species. Such events could have occurred during the long branch that is basal to the *Amphiprion* crown group. Also, it has been suggested that elevated extinction rates in reef associated fishes could be linked with a potential refuge effect of the coral reefs in the aftermath of prolonged extinction events [41]. Yet, to be able to recover extinction rate with high confidence and test such hypotheses, one would need clownfishes fossils [66], which are not available. However, when compared to the damselfishes, the diversification rate of the clownfishes was still higher (Figure 3), showing that the extinction rate was not sufficient to slow down diversification [41].

Effect of mutualism and host-associated niches on clownfishes evolution

We measured the phylogenetic signal of each morphological trait on the samples of chronograms and phylograms for the Pomacentridae (Tables 2 and 3). All traits showed a signal close to one (the expected

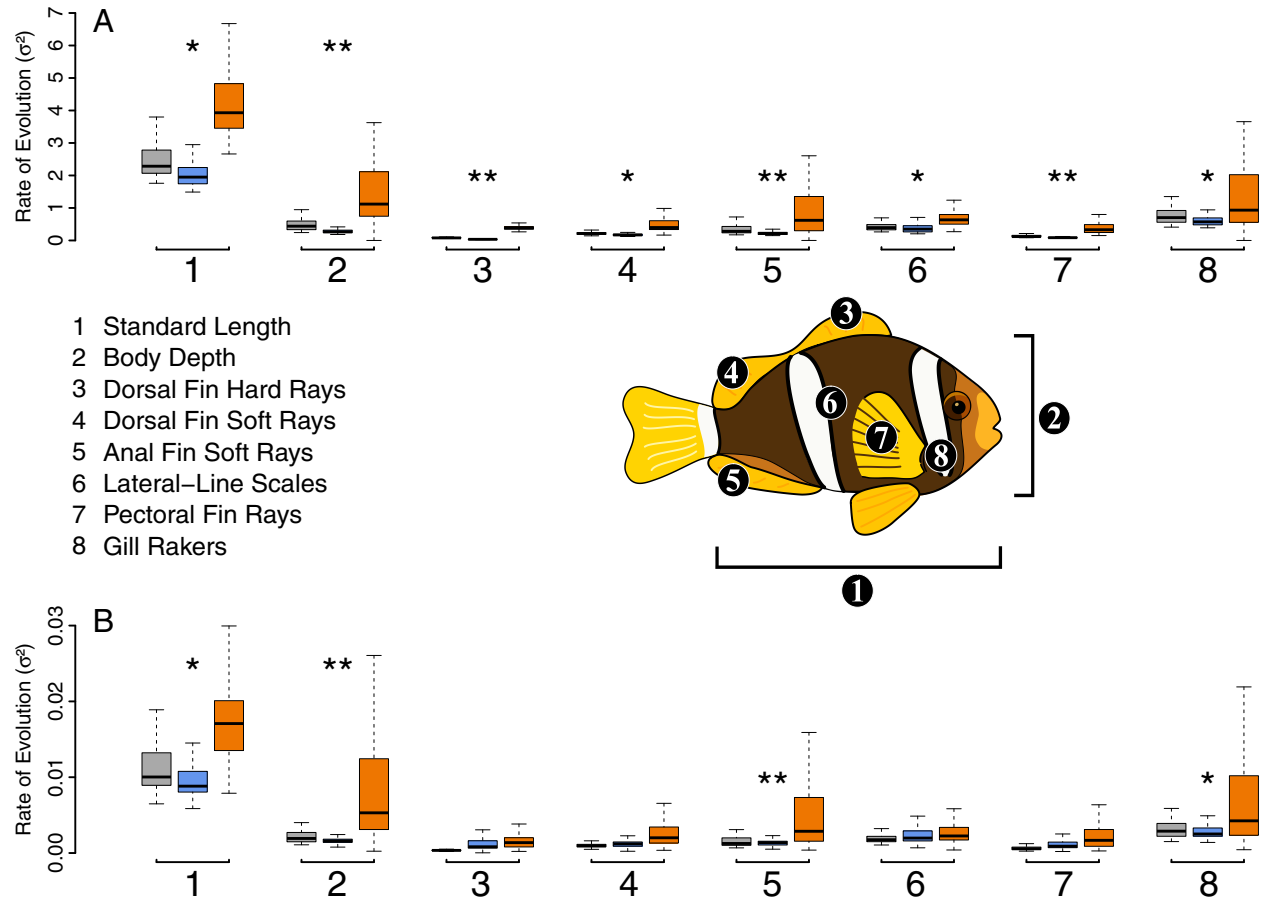

Figure 6 ***Rate of morphological evolution.*** Evolution of the morphological traits measured on the sample of 100 phylograms **(A)** and chronograms **(B)**. Rates of clownfishes are in orange, damselfishes in blue and the one-rate model is shown in white. The P-values of model comparison by likelihood ratio test is signified by asterisk (* = P-values <0.05, ** = P-values <0.01). The schematic position of each morphological trait is shown on the clownfish drawing.

outcome of BM evolution). However, when assessed only on the clownfish clade, phylogenetic signal dropped and only pectoral rays count had a K significantly larger than 0. Furthermore, the phylogenetic signal of the host usage MCA axes were never significantly different from 0. While interpreting an evolutionary process directly from this result can be problematic [67], a low phylogenetic signal can be found in lineages that show convergent adaptive evolution [68], which is likely the case in the clownfishes.

We hypothesised that, following the appearance of mutualism, clownfishes radiated in the niches associated with the sea anemones. We described the most important axes of variation in mutualistic interactions with an MCA. The first axis depicted the generalist-specialist host usage gradient, but all three other axes showed gradients linked with the habitat preferences of the sea anemones. Indeed, clownfishes that interact with sea anemones species living in similar reef micro-habitats (i.e. substrate type, depth) cluster together in the analysis. This suggests that clownfish species are first distributed along a generalist to specialist axis, and then, specialist clownfishes interact only with sea anemone species living in a particular habitat type. This has been shown in a previous empirical study [18], where clownfish species coexisting in a reef were distributed in different habitats. Ecological sorting of clownfish species along the different ecological gradients linked with their hosts is what is expected if resource competition, which is the main driver of adaptive radiation, acted on the evolutionary process [4,69].

We tested if the observed resource partitioning in different ecological niches, likely due to past competition between ecologically similar species, resulted in morphological adaptation to resource use (i.e. host and habitat use in clownfishes). We sequentially fitted each MCA axis to a set of morphological traits taking into account the phylogenetic relationships between species. We found that an important part of the variation in the MCA axes could

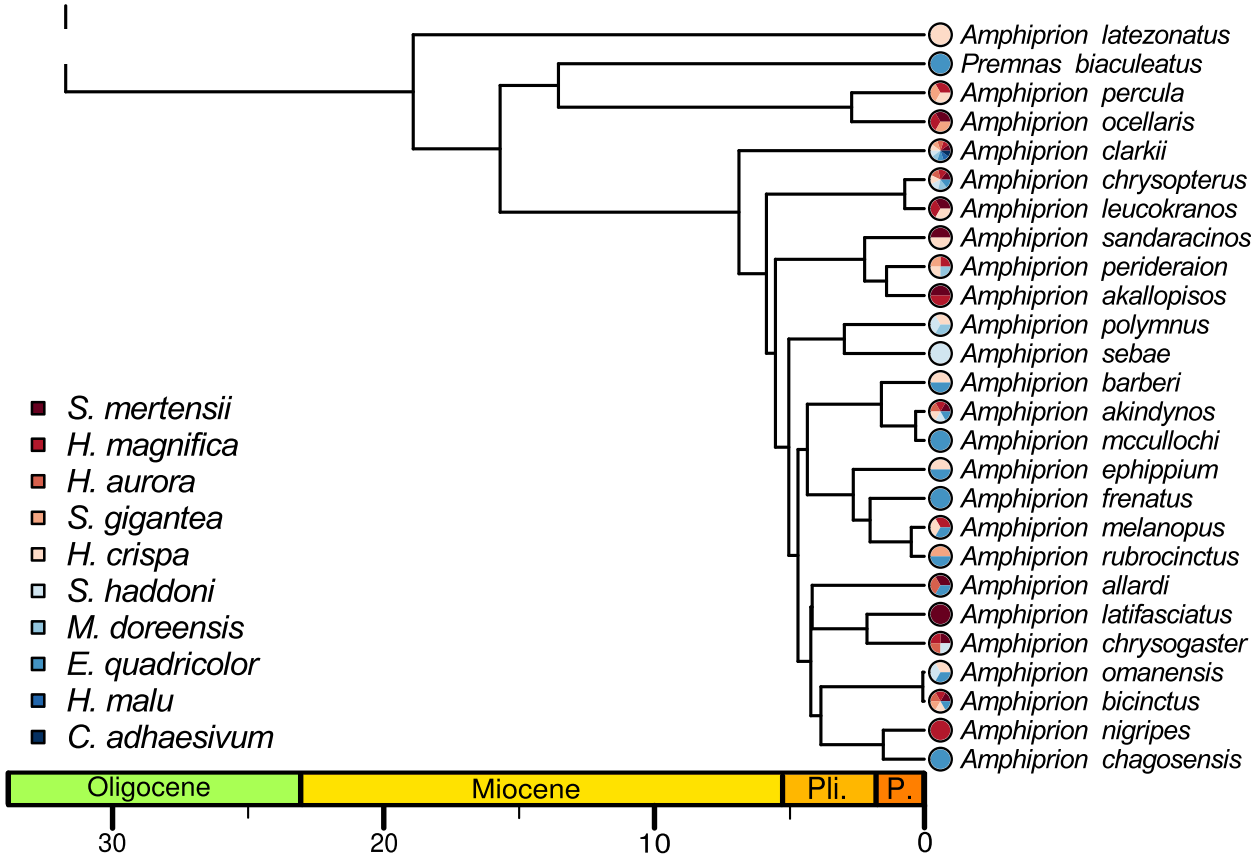

Figure 7 ***Chronogram of the clownfishes radiation.*** Branch lengths are given in MY. The interacting sea anemone species are shown for each clownfish species. Sea anemone names abbreviations as in Figure 5.

be explained by the morphological traits of our dataset for the first and third axes (Table 4). Increasing number of hosts (represented by increasing values of the MCA 1 axis) was correlated with a bigger size, more hard dorsal and soft anal rays, and more gill rakers, while size was positively correlated with deeper habitats (represented by decreasing values of the MCA 3 axis). In clownfishes, the phenotype-environment correlation relates to both host usage (generalists/specialist gradient) and habitat (substrate, depth). This contrasts with many examples of adaptive radiation, where the resource axis has usually a single dimension representing habitat. Therefore, mutualism can be seen as a a key innovation that offered untapped habitat for colonisation, but also allowed diversification to happen on the host usage resource axes.

The morphological traits studied are primarily used for taxonomic purposes but they can still give important functional information for the evolution of the clownfishes. Size and fin traits are related to the locomotion ability in various types of water velocities, while gill rakers can be used as a proxy for the trophic level. The picture given by our analyses is that generalist clownfishes (e.g. *A. clarkii*) will likely eat more planktonic food (and thus have more gill rakers) and be better swimmers than specialists, which never leave the close vicinity of their sea anemone host. Clownfishes that interact with sea anemones occurring at deeper depth have also a bigger size, allowing for better locomotion in areas where the water velocity is likely to be higher compared to more shallow and sheltered zones [70]. While our results show correlations between phenotype and environment, we do not test for trait utility. This would require a strict experimental setting that was out of scope for this paper. More studies are definitely needed to better describe the adaptive advantage that those traits may provide in the ecological context of the mutualistic interaction.

Following an ecological opportunity, the rate of morphological evolution is hypothesised to be elevated in the traits that are functionally related to the ecological niches filled during the radiation process [4,71,72]. We tested this hypothesis on the eight morphological traits studied and found that, on phylograms, they all evolved at higher rates in clownfishes than in damselfishes (Figure 6). The picture is similar when rates

are measured on chronograms, although only four characters are evolving significantly faster in the clownfishes than in the damselfishes in this case. Following the comparison of phylogenetic signal that was made between phylograms and chronograms, phylograms are assumed to give, in this case, more accurate results [51]. The fact that all traits evolve at a higher rate is congruent with the pGLS results, which shows all but two traits (lateral-line scales and body ratio) being significantly linked with host and habitat usage. It is probable that lateral-line scales and body ratio evolutionary rates were accelerated in clownfishes because they are indirectly correlated to the ecological niche through another trait. Thus they would not be themselves correlated to host-usage/habitat but still show accelerated rates of evolution. A clear followup to this broad description of clownfishes morphologies would be to extend our analysis and use a morphometric approach (e.g. [36]) to be able to give an accurate description of the different clownfishes ecotypes. Further studies will then be needed to test, in an experimental framework, trait utility, which is one of the diagnostic criteria of adaptive radiation [4]. The chemical biology of the interaction between clownfishes and sea anemones is also far from being solved [22]. It is thus possible that unknown characteristics associated for example, with the clownfishes mucus could be linked with the variation in interaction between clownfishes and specific sea anemones.

We did not take into account the distribution of the species in this study. Geographical isolation, coupled with ecological differentiation, could also be at the origin of the evolutionary pattern found here. While several clownfish species are local endemics that likely originated through vicariance events rather than ecological speciation, the majority of the species (17 out of 30) have overlapping distributions centred on the Indo-Malay archipelago. If reproductive isolation was solely due to geography, the latter species would have likely disappeared through hybridisation, which easily happens in captivity [73]. Moreover, clownfishes that have similar MCA values usually do not overlap in geographical distribution (e.g. *A. latifasciatus*, *A. nigripes* and *A. sandaracinos* in Figure 4), and sister species always differ in host usage as can be seen on Figure 7. Such a pattern could indicate that, in a given biogeographic region, only one species per ecological niche can subsist, but also that ecologically similar species evolved independently in geographically separated areas. This outlines the need for a thorough biogeographic analysis that would help clarify the effect of geography on the evolution of the clownfish.

Conclusion

Our study shows that clownfishes likely experienced an adaptive radiation through ecological speciation. The obligate mutualism with sea anemones is thought to be the key innovation that allowed clownfishes to radiate rapidly in untapped ecological niches. As expected under the ecological theory of adaptive radiation [4], it increased diversification as well as rates of morphological evolution. Clownfishes experienced rapid and convergent morphological changes that were correlated with the different ecological niches offered by the host anemones. In marine environments, barriers to dispersal are uncommon, which makes ecological speciation less likely than in more isolated landscapes [25]. However clownfishes show a very short dispersal period compared to other damselfishes [74]. In conjunction with a high retention of larva to natal reef [27] and population specific calls [30], restricted dispersal likely reinforced reproductive isolation between clownfish species allowing for adaptive radiation.

Additional files

Additional file 1: References of morphological data used in this study.

Additional file 2: GenBank accession number of the sequences used in this study.

Additional file 3: Substitution model choice.

Additional file 4: Phylogeny of the damselfish with branch lengths given in expected number of substitutions per site.

Additional file 5: Factorial map of the MCA analysis with eigenvectors for the axes 1 and 2.

Additional file 6: Factorial map of the MCA analysis with eigenvectors for the axes 3 and 4.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

GL and NS conceived the study. GL, CS and RW performed the analyses and drafted the manuscript. NS supervised GL, coordinated the project and helped to draft the manuscript. PB and NZ supervised RW, participated in the coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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APPENDIX IV SMALL AND UGLY. THE LOSS OF HETEROMORPHIC SELF-INCOMPATIBILITY IN PRIMROSES AND THE "SELFING SYNDROME"

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One of the most common transitions in plant evolution, the shift from outcrossing to increased selfing after the loss of self-incompatibility, is typically associated with changes in multiple floral characters, termed the selfing syndrome, notably including a reduction of floral size. However, it is unclear what aspects of evolutionary trajectories of floral morphology (e.g. inferred selective optima) change with a shift toward increased selfing and whether there are differences among traits. Here, we use recently developed comparative methods to study quantitative effects of losses of self-incompatibility on four floral traits, as exemplified by nine independent transitions from heterostyly to homostyly among 126 Primrose species, a classic system for the evolution of selfing. We find similar variability among heterostylous and homostylous flowers, but contrasting patterns among traits: homostylous flowers are smaller in some but not all respects. Patterns in pollination-related traits are best explained by a marked increase in the intensity of stochastic fluctuations of evolutionary trajectories associated with losing heterostyly, contradicting the general assumption that floral-morphological changes in selfing species are primarily driven by shifted optima of resource allocation. These results are congruent with an increased importance of drift for evolutionary trajectories of floral morphology after the loss of self-incompatibility.

Introduction

The loss of self-incompatibility (i.e., postpollination prezygotic mechanisms that prevent self-fertilization; Igic et al. 2008) is widely acknowledged as one of the most frequent transitions in plant evolution (Stebbins 1950, 1970). Furthermore, it has important implications for micro-evolutionary processes (Igic et al. 2008) and macro-evolutionary patterns of clade diversification (Takebayashi and Morell 2001; Goldberg et al. 2010; Ferrer and Good 2012; Chapter 2, this thesis). Much of the evolutionary significance of the loss of self-incompatibility relates to the notion that its loss is the prerequisite for the transition from allogamous (outcrossing) to predominantly autogamous (selfing) mating (Stebbins 1970; Barrett 2002; Busch and Schoen 2008; Wright et al. 2008; Karron et al. 2012; Raduski et al. 2012). While self-incompatible flowers are necessarily outcrossing, self-compatible flowers can either outcross, self or have an intermediate selfing rate, but high rather than low selfing is more common for self-compatible taxa (Raduski et al. 2012). Commonly, transitions toward increased selfing after the loss of self-incompatibility are associated with a suite of changes in morphological and reproductive floral characters (Darwin 1876, Ornduff 1969, Stebbins 1970), including a decreased floral display, a reduced pollen-to-ovule-number ratio, a smaller distance between male and female organs within flowers (i.e. less herkogamy) and a general reduction in floral size, collectively termed the "selfing syndrome" (see Table 1 in Ornduff 1969; Cruden 1977; Ritland and Ritland 1989; Goodwillie et al. 2010; Sicard and Lenhard 2011).

The selfing syndrome is considered a common phenomenon; transitions from outcrossing to increased selfing are thought to be "in most cases" (Sicard and Lenhard 2011) if not "almost universally" (Foxe et al. 2009) associated with the selfing syndrome. Stebbins (1970, p. 310) stated in an early discussion that "in all self-fertilizers, flower size diminishes below that found in their cross-fertilizing ancestors", suggesting that evolution toward a selfing syndrome upon the loss of self-incompatibility is a unidirectional, deterministic evolutionary trend. Yet, most of our understanding of the evolution of floral traits after the loss of self-incompatibility stems from explicit analyses on a few selected taxa (e.g. *Capsella*, Slotte et al. 2010; *Eichhornia*, Vallejo-Marín and Barrett 2009; *Leavenworthia*; Busch and Urban 2011; *Mimulus*, Ritland and Ritland 1989), or from informal interpretation of data on large numbers of species (e.g. Darwin 1876; Ornduff 1969; Stebbins 1970). Few comparative studies involving a larger number of species in an explicit phylogenetic framework have been

conducted (but see Goodwillie et al. 2010 for an angiosperm-wide analysis of floral display in inflorescences and selfing rates). Specifically, although multiple independent losses of self-incompatibility are documented in several clades (e.g. *Linanthus* section *Leptosiphon* (Polemoniaceae), Goodwillie 1999; Solanaceae, Goldberg et al. 2010; Triticeae (Poaceae), Escobar et al. 2010), it is unclear whether, or to what extent, replicate transitions in different species within a clade lead to similar evolutionary trajectories. Are the floral displays of self-compatible species always smaller than their self-incompatible relatives, as Stebbins (1970) suggested? Do individual floral traits respond differently to increased selfing? Do different floral traits evolve synchronously or asynchronously to the loss of self-incompatibility? These questions were identified as "unsolved mysteries in the transition to self-fertilization" (Karron et al. 2012) and are addressed in the current study.

Several reasons that are not mutually exclusive have been proposed for the correlation between decreased floral size and increased selfing rates (Sicard and Lenhard 2011). First, small floral size may facilitate autonomous selfing and be directly targeted by selection, for instance when selfing provides reproductive assurance under mate- or pollinator-limited conditions (Eckert et al. 2006). Second, if reproductive fitness is decoupled from the attractiveness of floral display for pollinators, as is the case in strict selfers, theory predicts that resources would not be invested in large flowers, but rather in increased reproduction (e.g. ovule production; Brunet 1992). Third, the selfing syndrome may be a pleiotropic effect of selection for small flowers driven by selection for the avoidance of herbivory (Eckert et al. 2006) or by selection for fast maturation in marginal habitats (Guerrant 1989; Aarssen 2000). These reasons suggest that after a transition toward increased selfing, floral size is under selection to progressively diminish in a range of scenarios.

Despite the broad acceptance of the selfing syndrome, the loss of self-compatibility does not necessarily result in small floral size. In fact, showy flowers with highly specialized pollination systems are often self-compatible and can have high selfing rates, in contrast with the prediction of the selfing syndrome (reviewed by Fenster and Martén-Rodríguez 2007). The occurrence of high selfing rates in showy, specialized flowers contradicts the interpretation that showiness is the product of selection for high maternal outcrossing rates (Fenster and Martén-Rodríguez 2007). This conflict may be explained by the idea that showy, specialized flowers, relying on a small subset of the potential pollinator community, are inherently prone to reproductive failure, and thus selfing may assure reproduction when outcrossing fails. Therefore, Fenster and Martén-Rodríguez (2007) concluded that a showy

floral display and pollinator specialization may evolve due to selective forces independent of those operating on the selfing rate. The notion that floral display may evolve independently from the selfing rate (Fesnter and Marten-Rodriguez 2007) implies that the loss of self-incompatibility does not necessarily lead to small flowers, as predicted by the selfing-syndrome theory. It is thus topical to ask whether floral traits respond to the loss of self-incompatibility consistently across different species.

In this study, we assess the extent to which the loss of self-incompatibility and the associated possibility of increased selfing results in a unidirectional, deterministic evolutionary trajectory towards smaller floral size, as predicting by the selfing syndrome, using the primroses as our study system. This group of ca. 550 species (i.e. the clade "/Primula" sensu Mast et al. 2001, that is, *Primula* and nested genera, Primulaceae; Richards 2003; see also Chapter 2, this thesis) is a classic model for the evolution of selfing, discussed in the seminal works of Ornduff (1969) and Stebbins (1970) as an example of a clade demonstrating the repeated loss of self-incompatibility and associated origin of selfing lineages, in the form of transitions from heterostyly to homostyly. Heterostyly is a form of heteromorphic self-incompatibility in which populations consist of two (distyly) or three (tristyly) genetic morphs that differ in their reciprocal placement of sexual organs and in their mating type, so that only crosses between morphs show full fertility (reviewed by Ernst 1962; Ganders 1979; Barrett 1992; Wedderburn and Richards 1992; Barrett and Shore 2008; Cohen 2010; Naiki 2012). Homostylous species have only one floral morph (i.e., are monomorphic), are self-compatible and hence, self-fertilization is possible. Detailed phylogenetic studies concluded that the crown node of the clade /Primula was heterostylous and indicated several, deeply nested losses of heterostyly within the clade (Mast et al. 2006, De Vos et al. 2012). Similar patterns occur in many of the ca. 28 plant families with heterostyly, with homostylous species evolving multiple times independently from heterostylous ancestors (besides in *Primula* e.g. in *Amsinckia*, Boraginaceae, Schoen et al. 1997; *Narcissus*, Amaryllidaceae, Graham and Barrett 2004; *Nymphoides*, Menyanthaceae, Tipperey and Les 2011; Pontederiaceae, Kohn et al. 1996; *Turnera*, Turneraceae, Truyens et al. 2005). The recurrent transition from heterostyly to homostyly is an important model for the evolution of selfing in angiosperms (reviewed by Barrett 2003), making it an ideal system to evaluate the selfing syndrome from a quantitative, comparative perspective.

Here, we analyze a large data set of multiple, quantitative floral traits in /Primula in a phylogenetic framework by using a combination of recently developed methods that employ

explicit models of quantitative trait evolution and account for both evolutionary relationships and intraspecific variation. We ask the following questions: Do heterostylous and homostylous species differ in (i) overall floral morphology and (ii) individual floral traits? (iii) How does the evolutionary trajectory (e.g., the inferred selective optimum) of each floral trait change upon the loss of self-incompatibility? By answering these questions, our study contributes to an understanding of the phenotypic consequences of the loss of self-incompatibility, one of the most important evolutionary transitions in flowering plant evolution.

Methods

Phylogeny

In this study, we used the 265-taxon, time-calibrated phylogeny of Primulaceae s.str. (Primulaceae subfamily Primuloideae, sensu Angiosperm Phylogeny Group 2009) estimated by De Vos et al. (Chapter 2, this thesis). Taxon sampling was designed to cover the morphological variation in the family, by including species from all genera and all sections, representing ca 35% of extant diversity. Phylogenetic relationships were inferred from four chloroplast markers using the uncorrelated lognormal relaxed clock method in BEAST v.1.6.2 (Drummond and Rambaut 2007). We calculated the maximum clade credibility (MCC) tree with median node heights for the /Primula clade (*Primula* and the nested genera *Dionysia*, *Cortusa* and *Dodecatheon*) from 1000 samples from the posterior distribution of phylogeny estimates for Primulaceae of De Vos et al., after pruning all species outside of /Primula from each sample. Subsequently, we removed branches from the MCC tree representing species for which no quantitative floral data was available (see below).

Morphological Data

For the designation of a species as heterostylous we relied on the accounts in "Flora of China" (Hu and Kelso 1996), Richards' (2003) comprehensive monograph of *Primula*, Grey-Wilson's (1989) account of *Dionysia*, and the extensive review by Ernst (1962). For analyses that did not account for intra-specific variation, we followed Ernst (1962) in scoring a species' predominant breeding for species with multiple breeding systems reported.

Quantitative floral measurements were assembled from three sources. First, detailed data on the floral morphology of *Primula* species were meticulously reported in the series "Stammesgeschichtliche Untersuchungen zum Heterostyly-Problem" by Ernst (1938, 1949,

1953, 1956, 1959, 1961, 1962) for a total of approximately 835 pages. These data, consisting of ten measurements on each of 2680 flowers representing 138 currently accepted species, were digitized using Optical Character Recognition software (Readiris Pro v.11, I.R.I.S. Group S.A., Louvain-la-Neuve, Belgium) on high-resolution scans, manually corrected, and proof-read twice. We followed the most recent comprehensive monograph of *Primula* for species synonymy (Richards 2003). Secondly, we extracted ranges and means of the respective floral traits from "Flora of China" (Hu and Kelso 1996) for the ca. 300 Chinese *Primula* species. The ranges listed in this treatment are differentiated between heterostyly and homostyly and typically stem from observations on multiple herbarium sheets per species in multiple herbaria (pers. comm., S. Kelso), ensuring that intraspecific variation is adequately captured. Finally, for the species of *Dionysia*, we used the information provided in the monograph of Grey-Wilson (1989). We did not include measurements from species of the nested genus *Dodecatheon,* because their aberrant floral structure (Mast et al. 2004) impedes meaningful quantitative comparisons of the size of floral organs to other species in the clade.

Among the available floral measurements, we selected four floral traits that are thought to influence a plant's mating system: the distance from the base of the flower to (a) the apex of the calyx (i.e., calyx length) and (b) to the mouth of the corolla-tube (i.e., tube length), (c) the diameter the corolla limb (i.e., corolla diameter), and (d) the absolute distance between the top of the male (anthers) and female (stigmas) organs within flowers (i.e., herkogamy). We included the compound trait herkogamy rather than the position of anthers and stigmas separately, because it is problematic to compare anther and stigma positions of species with and without heterostyly. Moreover, herkogamy has been shown to affect the genetic selfing rate (e.g., Herlihy and Eckert 2007) and the probability of autonomous self-fertilization (De Vos et al. 2012) and is therefore a more meaningful to compare between heterostylous and homostylous species than the absolute position of sexual organs. Some floral characters for which data was available, for ex., the length of the calyx teeth or the degree of incision of the corolla lobes, were excluded from further analyses, because we expected strong correlations with the traits we included. For analyses not accounting for intra-specific variation, we calculated the means of the four traits listed above in all the 126 species of the phylogeny by De Vos et al. (Chapter 2, this thesis) for which data were available.

Ancestral State Inference

To assess the number of independent losses of heterostyly captured by the taxon sampling of the current study, we inferred the presence/absence of heterostyly at ancestral nodes in a

likelihood framework, using the function ace in the R-package ape (Paradis et al. 2004). We calculated the likelihood of the data under the alternative models of equal rates of gain and loss (the SYM model) and different rates of gain and loss (the ARD model) using each of 1000 trees of the posterior distribution of trees from which the MCC tree was calculated. We assessed model fit based on the distribution of AIC scores and calculated the likelihoods associated with presence/absence of heterostyly at all ancestral nodes in the MCC tree.

Floral Differentiation

To quantify the extent of phylogenetic signal in all four traits, we used Pagel's (1999) lambda, a scaling parameter of the off-diagonal elements of the phylogenetic variance-covariance matrix, as implemented in the R-package geiger (Harmon et al. 2009), because this measure performed comparatively well among a set of estimators of phylogenetic signal (Münkemüller et al. 2012). We determined if lambda was significantly different from both zero and one using likelihood-ratio tests. To summarize quantitative variation and covariation of all floral traits among species with and without heterostyly, we performed a phylogenetic principal component analysis using the function phyl.pca in the R-package phytools (Revell 2012) on mean values per species and trait (Revell 2009). We employed the appropriate scaling factor for branch lengths determined by the test for phylogenetic signal.

To test whether individual floral traits differ between species with and without heterostyly, we used four generalized linear mixed models, one for each floral trait, implemented in the R-package MCMCglmm (Hadfield 2010), which accounts for both intraspecific variation and phylogenetic relatedness of species. We used "presence of heterostyly" as predictor variable, fitted a univariate normal response to the data of each floral trait, and included phylogeny and intraspecific variation as random variables. Models were run for 2,500,000 iterations with a burnin of 1,000,000 iterations and a thinning interval of 1000 iterations. We adjusted the standard, weak priors to facilitate convergence by splitting the observed total variance in our response variables in equal parts between the random (phylogenetic and intra-specific) and the residual variance components. We assessed the significance of the predictor's effect by determining if the 95% credible interval (95% CI) of the effect size (i.e. the difference between intercepts) included zero.

Models of Trait Evolution

To test whether the evolutionary trajectories (see below) of floral traits differ between heterostylous and homostylous species, we fitted a series of likelihood models for continuous

characters and compared the estimated parameters among the most likely candidate models. To this end, we modeled quantitative trait evolution as a Ornstein–Uhlenbeck (OU) stochastic process, which describes a combination of random drift (termed Brownian Motion; BM) and a deterministic, selective "pull" toward an optimal value, termed θ (Hansen 1997; Butler and King, 2004; Beaulieu et al. 2012). The evolution of the trait toward θ is governed by a constant describing the strength of selection, termed α, and a constant that measures the intensity of drift-like random fluctuations in the evolutionary process, termed σ^2. When α=0, the model collapses to BM (hereafter BM_1); when α>0, the model is termed OU_1, where the subscript "1" refers to the presence of a single, global optimum θ. Although these models employ terms similar to those used for micro-evolutionary processes (e.g. drift, selection), they actually describe the pattern of evolutionary change, i.e. the evolutionary trajectory (Beaulieu et al. 2012). To avoid confusion, we make an explicit distinction between genetic drift, which is a population-genetic process, and macro-evolutionary drift, described by the model-parameter σ^2, throughout the paper.

Recently, these models of quantitative trait evolution have been generalized to incorporate multiple values for θ, α, and/or σ^2 that can be associated with the evolution of discrete character-states along the phylogeny (Butler and King 2004, O'Meara 2006, Beaulieu et al. 2012). The mapped history of a character, heterostyly in the current context, divides the phylogeny in heterostylous and homostylous partitions; θ, α, and/or σ^2 are then fitted to the quantitative data with global or partition-specific values. By comparing support for models that either have single or multiple values for θ, α, and/or σ^2, we can thus determine which aspects of the evolutionary trajectory change with the loss of heterostyly (Table 1).

We considered five models with multiple θ, α and/or σ^2. The BM_S model includes one global, optimal trait value θ, but the intensity of the stochastic fluctuations, σ^2, can differ along the phylogeny as determined by the presence or absence of a character-state (O'Meara et al., 2006). The OU_M model, with two θ but one α and one σ^2, describes the situation where a floral trait may evolve toward different optimal values, for instance indicated by a smaller θ for homostylous than heterostylous species, while the rate of evolution towards these optima is the same (Butler and King 2004). Beaulieu et al. (2012) recently implemented expanded OU_M-models in which, besides θ, also α or σ^2 varies with the character history (i.e. OU_{MA} and OU_{MV}, respectively). In the most general case, θ, α and σ^2 are each estimated separately for heterostylous and homostylous tree partitions (the OU_{MVA} model; Beaulieu et al. 2012).

Table 1 Models of quantitative-trait evolution relevant to this study with their parameters and biological interpretation, indicating for each model whether the optimal trait value, θ, the intensity of random fluctuations in the evolutionary trajectory, σ^2, and the selective "pull" toward the optimal value, α, is modeled with one global parameter or with two parameters that are heterostyly- and homostyly-specific.

Model	Parameters			Interpretation for quantitative trait evolution
	Theta	***Sigma²***	***Alpha***	
BM_1	Global	Global	-	Evolution is random and not affected by the loss of heterostyly
BM_S	Global	State-specific	-	Evolution is random but the loss of heterostyly affects the rate of change
OU_1	Global	Global	Global	Evolution is directed toward an optimum value without being affected by the loss of heterostyly
OU_M	State-specific	Global	Global	The loss of heterostyly is associated with a shift toward a different optimal value
OU_{MA}	State-specific	Global	State-specific	The loss of heterostyly is associated with a shift toward a different optimal value that exerts a different selective pull
OU_{MV}	State-specific	State-specific	Global	The loss of heterostyly is associates with shifts toward a different optimal value and in the rate of random change

Implementation

To compare how well these seven models (two models with global and five models with multiple values for θ, α and/or σ^2, respectively) fit the floral-trait data of heterostylous and homostylous species, we first assigned each species to either breeding system. We then used stochastic character mapping (Huelsenbeck et al. 2003) implemented in the R-package phytools (Revell 2012) to sample 100 possible histories of the loss of heterostyly given the maximum likelihood estimate of the rate of change in presence of heterostyly. We followed Mast et al. (2006) in using the “equal-rates” transition model (i.e. SYM) for the evolution of heterostyly in /Primula, but also tested the “all-rates-different” transition model (i.e. ARD). We chose to use stochastic maps, rather than the maximum likelihood estimate, to allow for incorporation of uncertainty in the evolutionary history of heterostyly in the estimation of differences between the evolutionary trajectories of floral traits of heterostylous and homostylous species, a strategy that was found to be useful in other studies (e.g. Price et al. 2012).

Models were fitted using the R-package OUwie (Beaulieu et al. 2012). To facilitate model fitting, we divided all trait values by ten and adjusted the initial values of the likelihood search, trying values of 0.01, 0.3, or 1.0. Nevertheless, for most mapped histories, it was

impossible to fit the most complex model, OU_{MVA}, to the data. Inspection of the eigendecomposition of the Hessian matrix and examination of the eigenvectors, as recommended by Beaulieu et al. (2012), revealed that problematic inference was usually related to difficulties in estimating α jointly with σ^2 from the data. Therefore, OU_{MVA} models were considered too complex for the information contained in the data and abandoned (Beaulieu et al. 2012). We also excluded mapped histories for which the maximum likelihood could not be determined reliably in all models as indicated by negative eigenvalues of the Hessian (Beaulieu et al. 2012). Model fit was determined using AICc weights calculated from $\triangle$AICc scores (Burnham and Anderson 2002). AICc weights can be interpreted as the probability that a model is the best one among the candidate models. We considered models with AICc weights < 0.05 to be not supported by the data and all models with AICc weight > 0.05 to be plausible. As advised by Beaulieu et al. (2012), we interpret results by comparing differences in parameter estimates between heterostylous and homostylous species among the set of plausible models for the evolution of each trait.

Expectations

The selfing-syndrome theory predicts that flower size should be lower in homostylous than heterostylous species. Therefore, models with two optima, θ (OU_M, OU_{MA}, and OU_{MV}) are expected to receive higher AICc weights than models with one θ (BM_1, BM_S, OU_1) and the inferred θ should be smaller for homostylous species. Secondly, selfing is generally expected to lead to lower effective population sizes, which in turn implies that genetic drift becomes more important in the evolutionary process (Lloyd, 1980; Hamrick and Godt, 1996). Thus, floral trait evolution is expected to be more stochastic in homostylous than in heterostylous species, because homostylous species are likely to have higher selfing rates than heterostylous species. Therefore, we predict high AICc weight for the model that allows for two σ^2 (OU_{MV}), with higher σ^2 for floral traits of homostylous species. Finally, the self-compatible flowers of homostylous species rely less on providing an adequate fit to their pollinator(s) for reproduction than obligately outcrossing, heterostylous species. Consequently, homostylous species are expected to be less affected by selective constraints imposed on floral traits by pollinators than heterostylous species. Instead, homostylous species are expected to experience stronger selection for low herkogamy to facilitate self-fertilization. Therefore, we predict that the model with two α (OU_{MA}) receives high AICc weight for all traits. In addition, we predict that homostylous species have a higher α for herkogamy, but lower α for other traits, compared to heterostylous species.

Results

Ancestral State Inference

The likelihood of the data given an asymmetrical model of character evolution (ARD) was higher than under the less complex, symmetrical model (SYM), as reflected in lower AIC scores (ARD: 85.47±0.05; mean AICc among all trees ±1 SE; SYM: 99.59±0.07). However, the ARD model inferred a 9-fold higher rate of gain than loss of heterostyly (q_{01}: 0.425±0.004; q_{10}: 0.047±0.001), which seems highly unrealistic and could be an artifact. The inference of a high forward rate q_{01} appears to be driven by the presence of 2-3 implied re-gains of heterostyly over short branches (*Primula farinosa, P. aurantiaca,* and perhaps *P. pulverulenta*), but the particular topological relationships are not well supported and partially in conflict with detailed studies at the sectional level (Guggisberg et al. 2006, 2009). After repeating the analysis without these three species, the ARD model was indeed no longer supported over the SYM model (not shown). Figure 1 illustrates the ancestral states at internal nodes of the MCC tree as the proportion of likelihood associated with presence/absence of heterostyly under the SYM model. The deeper nodes are significantly more likely to be heterostylous than homostylous; the distribution of states at the tips imply that our phylogenetic sampling captures nine independent losses of heterostyly, indicating that our data provides a good model for the repeated loss of self-incompatibility.

Floral Differentiation

All quantitative floral traits showed significant phylogenetic signal (corolla-limb diameter: $p=0.041$; other traits $p<0.001$). Values of Pagel's lambda were 0.826 (calyx length), 0.772 (tube length), 0.525 (corolla-limb diameter), and 0.778 (herkogamy). This justifies analysis and interpretation of the floral data in a phylogenetic context.

The phylogenetic principle component analysis produced four axes (PCs) that explained 50.4%, 20.8%, 15.9% and 12.9% of variance, respectively. PC 1 was negatively correlated with all traits (factor loadings between -0.59 and -0.71), whereas PC 2 was correlated strongly and positively with herkogamy (factor loading 0.72) and negatively with corolla-limb diameter (factor loading -0.49). The scatterplot diagram of PCs 1 and 2 showed that the PCA

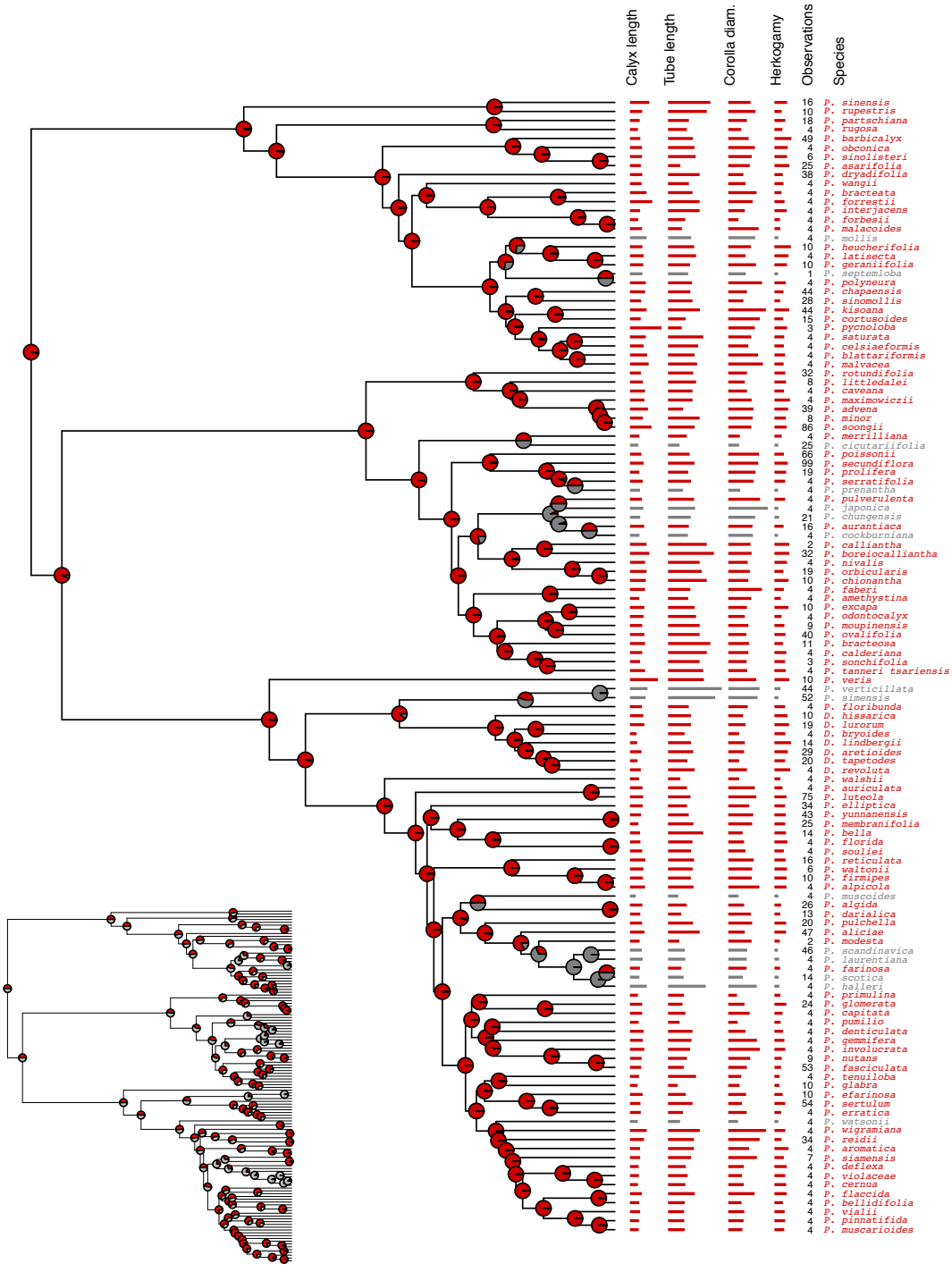

Fig. 1 Maximum clade credibility chronogram and characters states of the clade /Primula. Main figure: pie charts at internal nodes indicate the proportion of likelihood associated with the ancestral state being heterostylous (in red) and homostylous (in grey), based on the SYM-model, in which rates of losses and gains of heterostyly are constraint to be equal. Bars to the right of the tree are drawn with length proportional to the mean value in mm per species of the four analyzed floral traits (left to right: calyx length, corolla-tube length, corolla-limb diameter, and herkogamy), where red and grey bars are used for heterostylous and homostylous species, respectively. The column Observations indicates the number of observations that were available to calculate species means and account for intraspecific variation in the MCMCglmm analyses. Inset figure: phylogeny with pie charts indicating ancestral states as for the main figure, but based on the ARD-model, in which rates of losses and gains of heterostyly are estimated separately. Despite the apparent difference between the ARD and SYM ancestral state reconstructions, the results of downstream analyses were qualitatively the same.

scores of species with and without heterostyly largely overlapped on axis 1, but species without heterostyly had generally lower scores on PC 2 (Fig. 2).

The MCMCglmm analyses, which accounted for intraspecific variation and phylogenetic relatedness of species, indicated that all investigated floral traits were significantly different between homostylous and heterostylous species (Fig. 3). Although the 95% credible intervals (CIs) of posterior means of heterostylous and homostylous species overlapped considerably (Fig. 4), the CIs of the effect size of homostyly (i.e. the relative difference in size of traits in homostylous compared to heterostylous species) did not include zero for any trait (Fig. 3). The directionality of change differed among traits, as the sign of the effect sizes differed among traits (Fig 3). In contrast with the expectations under the selfing syndrome, homostylous species tended to have longer corolla tubes and calyces than related heterostylous species (95% CIs of the effect size 0.08 to 1.07 and 0.62 to 1.07, respectively; Figs 3, 4AB). Congruent with the selfing syndrome, corolla-limb diameter and herkogamy tended to be smaller in homostylous species (95% CIs of the effect size -1.79 to -0.39 and -4.24 to -4.51, respectively; Figs 3, 4CD).

Models of Trait Evolution

The results of the model-fitting for six models of quantitative trait evolution are summarized in Table 2 as means across 100 stochastic maps with standard error and associated AICc weights. Results including models not supported by the data are given in Table S1 (Supplementary Information). We only report results based on stochastic maps simulated under the SYM model, as results based on the ARD model stochastic maps were qualitatively congruent. Quantitatively, differences between heterostylous and homostylous species were less pronounced under the ARD approach (Table S1).

The best model for calyx length was OU_M (AICc weight 0.42), where θ was slightly lower for homostylous than for heterostylous species (0.53 and 0.69, respectively), congruent with the MCMCglmm results and predictions of the selfing syndrome. Although OU_{MA} and OU_{MV} also received considerable AICc weight (0.16 and 0.17, respectively), estimates of σ^2 and α were similar between heterostylous and homostylous species, indicating that if there are differences at all, they are small.

The best model for corolla tube length was OU_{MV} (AICc weight 0.67). In contrast with Congruent with our predictions, θ was smaller for homostylous species then heterostylous

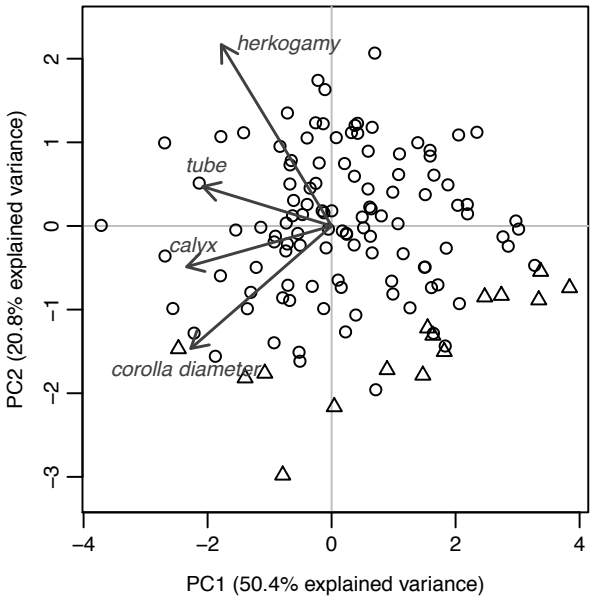

Figure 2 Scatterplot diagram of phylogenetic principal component analysis on four floral traits (calyx length, corolla-tube length, corolla-limb diameter, and herkogamy) for the first two principal components (PC1, PC2). Triangles and circles represent homostylous and heterostylous species, respectively. Arrows indicate factor loadings on PC1 and PC2. The two principal components together explain 71.18% of total variance among species.

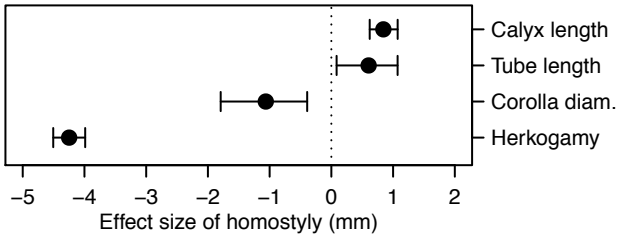

Figure 3 Posterior estimates of the effect size of homostyly on calyx length, corolla-tube length, corolla-limb diameter and herkogamy. Dots represent the mean of the posterior estimate with 95% credible intervals (CIs) represented by horizontal bars. Because the 95% CIs do not overlap with zero, all traits significantly differ between heterostylous and homostylous species: calyx and corolla tube are longer in homostylous species (positive effect size), whereas corolla-limb diameter and herkogamy are larger in heterostylous species (negative effect size).

species (1.28 and 1.52, respectively) and σ^2 was much higher in homostylous species (0.60 versus 0.16 in heterostylous species), which fits our prediction that macro-evolurionary drift is more important in homostylous species. The θ optima for both calyx and corolla tube lengths inferred by model fitting appeared to be incongruent with posterior estimates from MCMCglmm analyses, which inferred slightly higher mean trait values to homostylous species. The OUMA model received only half as much support (AICc weight 0.33) as the

OUMV model and indicated a higher α for homostylous than heterostylous species. This would be contrary to our expectations.

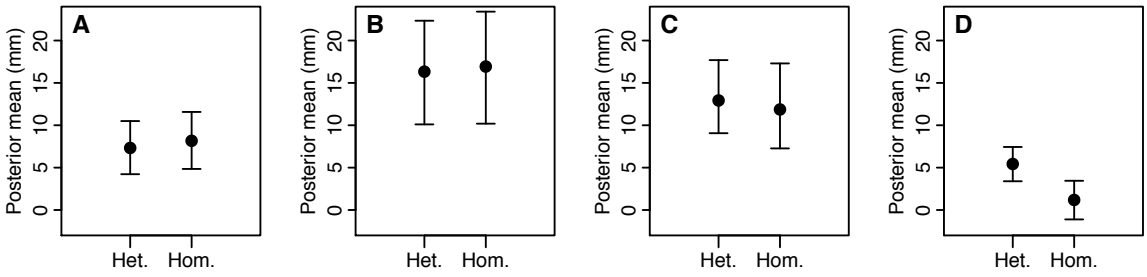

Figure 4 Posterior means of traits of heterostylous (Het.) and homostylous (Hom.) species in (A) calyx length, (B) corolla-tube length, (C) corolla-limb diameter, and (D) herkogamy inferred from the MCMCglmm analyses. Dots represent the overall meta-analytical posterior means with 95% credible intervals (CIs) represented by the vertical bars. Although the overall posterior means overlap strongly between heterostylous and homostylous species, the effect due to homostyly is significant for all traits (see also Fig. 3).

species (1.28 and 1.52, respectively) and σ^2 was much higher in homostylous species (0.60 versus 0.16 in heterostylous species), which fits our prediction that macro-evolurionary drift is more important in homostylous species. The θ optima for both calyx and corolla tube lengths inferred by model fitting appeared to be incongruent with posterior estimates from MCMCglmm analyses, which inferred slightly higher mean trait values to homostylous species. The OUMA model received only half as much support (AICc weight 0.33) as the OUMV model and indicated a higher α for homostylous than heterostylous species. This would be contrary to our expectations.

The diameter of the corolla limb was also best modeled under OU_{MV} (AICc weight 0.39). Interestingly, θ was nearly the same between heterostylous and homostylous species (1.28 and 1.27, respectively), but, congruent with the results of corolla tube length, σ^2 was again higher in homostylous species (3.70 versus 2.04 in heterostylous species), suggesting that higher levels of stochasticity affected the evolution of both traits after the loss of heterostyly. OU_M and OU_1 also received some support (AICc weight both 0.27), as did OU_{MA} (AICc weight 0.07), but both α were nearly the same under the latter model, making it effectively identical to the OU_M model.

For herkogamy, the OU_{MV} model received AICc weight of 1.00, indicating that it is the only plausible model. Congruent with the selfing syndrome, homostylous species had lower θ than

Table 2 Model fit and estimated parameters of plausible models (AICc weight > 0.05) for the four floral traits, indicating corrected AIC score (AICc), AICc weight, and the estimated values of the parameters θ (theta; optimum in cm), α (alpha, selective pull) and σ^2 (sigma^2; rate of random drift). When models contain a single, global parameter, estimates are italicized and printed in the center of the column; estimates for heterostyly- and homostyly-specific parameters are reported in their respective columns.

Trait	Model	AICc	AICc weight	*Theta*		*Sigma^2*		*Alpha*	
				Heterostyly-specific	Homostyly-specific	Heterostyly-specific	Homostyly-specific	Heterostyly-specific	Homostyly-specific
Calyx length	OU_1	12.642±0.000	0.247±0.006	*0.684±0.000*		*0.958±0.000*		*6.351±0.000*	
	OU_M	11.578±0.056	0.424±0.005	0.686±0.003	0.525±0.005	*0.047±0.000*		*0.313±0.000*	
	OU_{MA}	14.138±0.478	0.157±0.003	0.692±0.002	0.521±0.005	*0.045±0.000*		0.301±0.005	0.305±0.005
	OU_{MV}	13.427±0.091	0.172±0.007	0.686±0.004	0.522±0.005	0.046±0.001	0.055±0.001	*0.308±0.001*	
Corolla-tube length	OU_{MA}	201.951±1.555	0.321±0.025	1.566±0.008	1.333±0.009	*0.127±0.004*		0.179±0.014	0.219±0.013
	OU_{MV}	191.999±0.162	0.666±0.025	1.520±0.006	1.280±0.009	0.163±0.008	0.604±0.010	*0.268±0.000*	
Corolla-limb diameter	OU_1	173.267±0.000	0.269±0.005	*1.278±0.000*		*60.242±0.000*		*136.543±0.000*	
	OU_M	173.265±0.000	0.270±0.005	1.277±0.000	1.281±0.000	*2.944±0.002*		*6.674±0.004*	
	OU_{MA}	189.454±2.787	0.068±0.005	1.276±0.001	1.131±0.021	*0.359±0.019*		0.787±0.053	0.809±0.053
	OU_{MV}	172.511±0.080	0.393±0.008	1.278±0.000	1.274±0.002	2.041±0.073	3.692±0.126	*4.964±0.164*	
Herkogamy	OU_{MV}	-81.731±0.201	0.996±0.001	0.502±0.013	0.100±0.013	0.564±0.036	0.088±0.023	*7.845±0.428*	

Parameter estimates are reported as mean ± standard error across 100 stochastic maps generated using the SYM model for the evolution of heterostyly. See table S1 in the Supporting information for results including models not supported by the data (AICc weight <0.05) and for results based on stochastic maps generated under the ARD model.

heterostylous species (0.10 and 0.50, respectively). In contrast to the other traits for which OU_{MV} was the best model, σ^2 was lower in homostylous species (0.56 versus 0.09 in homostylous species).

Discussion

The commonly expected effect of the loss of self-incompatibility on the evolutionary trajectories of floral traits is a unidirectional, deterministic trend toward small floral size and shifted resource allocation (i.e., the selfing syndrome), mainly because species that self have smaller returns from investment in traits that attract pollinators (Sicard and Lenhard 2011). Our results are partially congruent with evolution toward smaller floral traits upon the loss of self-incompatibility (i.e. loss of heterostyly), as self-compatible species (i.e. homostyles) have smaller selective optima, θ, for all traits in the evolutionary models inferred to be most plausible (Table 2). However, our analyses also suggest a more complex, versatile evolutionary fate of self-compatible lineages. First, we find that homostylous species span a similar range of variation in overall floral morphology as heterostylous species, with the exception of herkogamy (compare PC 1 and PC 2 and their factor loadings, Fig. 2). Second, although our Bayesian glmm and evolutionary-model fitting analyses indicated that all floral traits differ between heterostylous and homostylous species (effect sizes do not include zero, Fig 3; strongest support for evolutionary models that differentiate heterostylous and homostylous species, Table 2), the posterior means of corolla-tube and calyx-length traits were significantly higher in homostyles, whereas corolla-limb diameter and herkogamy were significantly larger in heterostylous species (Fig. 3), indicating contrasting effects of the loss of heterostyly on different floral traits. Third, the best supported models for corolla-limb diameter and corolla-tube length are characterized by strongly different degrees of stochastic fluctuations during evolution, σ^2, with much higher stochasticity affecting trait evolution in homostyles (Tables 1, 2). Taken together, the variability and direction of change in the floral traits of homostylous species detected by our analyses contrast with the traditional paradigm of the selfing syndrome and with Stebbins' (1970) influential remark that selfing lineages *always* have smaller flowers than their outcrossing relatives.

The transition from heterostyly to homostyly is a classic system to investigate the genetic, ecological, and population biological contexts for the evolution of selfing (reviewed e.g. by Ernst 1955; Lewis and Jones 1992; Barrett 1992; Barrett 2003; Barrett & Shore 2008; Naiki 2012). Field experiments revealed a high capacity for self-fertilization in several homostylous

primroses (e.g. Washitani et al. 1994; Chen 2009; Carlson et al. 2010; De Vos et al. 2012), although genetic estimates of the actual selfing rate are rarely available (Piper et al. 1984). Our analyses indicate that homostylous species have strongly reduced herkogamy (Figs 3, 4) and a lower selective optimum, θ, for herkogamy, which was only slightly larger than zero (Table 2). Since herkogamy typically correlates negatively with the degree of auto-fertility (in e.g. *Primula*, De Vos et al. 2012; *Aquilegia*, Herlihy and Eckert 2007) the low - though non-zero - average herkogamy of homostylous species suggests that these species do not exclusively self (De Vos et al. 2012), but generally have selfing rates distinctly higher than self-incompatible heterostylous species. The transition from heterostyly to homostyly and the associated loss of self-incompatibility is thus a well-suited system for testing floral differentiation between outcrossing and (largely or partially) selfing species, but we note that it is possible that the ability of (some) homostylous species to reproduce both autogamously and allogamously plays a role in explaining the high variability of floral traits in homostylous species, in comparison to their obligately outcrossing, heterostylous relatives.

Although a general relationship between the evolution of polyploidy and the loss of heterostyly has been suggested for *Primula* (Richards 2003; Naiki 2012), comparision of ploidy levels (following Richards 2003) of sampled homostylous species with those of related, heterostylous species revealed that polyploidy unlikely is a major confounding factor in our analyses. For transitions involving homostylous species of known ploidy level, homostylous species usually were either exclusively diploids (*P. mollis, P. septemloba, P. simensis*+*P. verticillata*), or transitions to homostyly gave rise to a clade of diploid and polyploid species (*P. chungensis*+*P. cockburnia*+*P. japonica*) or to a polyploid (*P. watsonii*) that was sister to a clade containing both diploid and polyploid, heterostylous species. In one case, heterostylous, diploid species were sister to homostylous, polyploid species, but flower size of these homostylous species (*P. halleri, P. laurentiana, P. scandinavica,* and *P. scotica*) was either smaller or larger than that of related, heterostyous species (Fig. 1). Thus, our data show no strong relationship between the transition to homostyly and the evolution polyploidy, nor a trend of homostylous, polyploid species being larger or smaller than related diploid, heterostylous species.

While current analytical approaches do not allow us to discern whether the signal of strong macro-evolutionary drift, σ^2, in homostylous species (Table 2) reflects a high variability in selective optima, θ, among homostylous species or temporally fluctuating optima within and among homostylous lineages, they nevertheless enable us to conclude that homostylous

species display considerable phenotypic variation - more than predicted by the paradigm of selfing as being typically associated with reduced floral size in self-compatible lineages. Additionally, differential strength of selection among tree partions (i.e. multiple α) is generally difficult to detect when tree partitions span unequal amounts of evolutionary time (Beaulieu et al. 2012), as in the current dataset (Fig. 1), and co-estimation of multiple α and σ^2 proved not possible (see methods). Therefore, the higher levels of stochasticity, σ^2, detected by the two best models for the evolution of corolla-tube length and corolla-limb diameter after the loss of heterostyly (Table 2) suggest that the evolutionary trajectory of these traits is likely to include values that are more extreme in homostyles, irrespective of whether the microevolutionary process is driven by increased genetic drift, decreased selection, or a combination of both.

Because higher selfing rates, as expected for homostyles compared to heterostyles, will generally lead to lower effective population sizes, genetic drift is indeed likely to become stronger after a transition to homostyly (Lloyd 1980; Hamrick and Godt 1996). Moreover, the increased auto-fertility of homostylous compared to heterostylous species implies that homostylous species rely less on pollinators for successful reproduction. Therefore, pollinators would exert less stabilizing selection on the floral traits of homostylous species compared to heterostylous species (Cresswell 1998), an expectation congruent with the empirical finding that levels of floral integration may decrease after self-incompatibility is lost (Anderson and Busch 2006). Thus, changes in floral traits involved in pollinator attraction (e.g., corolla-limb diameter) and interaction (e.g., corolla-tube length) are likely to become more easily fixed by neutral processes in homostylous than in heterostylous species, a prediction that could explain the relatively wide variation of such floral traits in homostylous species (Figs 1, 2, 4). At the same time, the lower effective population size of selfing species further implies that the efficacy of selection should increase (Lloyd 1980; Hamrick and Godt 1996), meaning that achieving adaptation to a new fitness optimum may proceed faster in species without heterostyly (Glémin and Ronfort 2012). To summarize, both neutral and adaptive processes affecting the evolution of floral morphology are likely to proceed at a higher rate in homostylous compared to heterostylous species. Indeed, *Primula* species that have much longer floral tubes than other species of their section are usually homostylous (e.g. *Primula halleri* vs. other species of Section *Aleuritia*, or *P. verticillata* vs. other species of Section *Sphondylia*; Richards 2003). Moreover, multiple self-compatible lineages within the clade /Primula are morphologically so aberrant that they are frequently recognized as separate

genera (i.e. *Dodecatheon, Cortusa, Sredinskaya*), whereas this is the case for only one group of self-incompatible species (i.e. *Dionysia*; Scott 1865, Richards 2003, Mast et al. 2006, Reveal 2009).

A combination of of genetic drift and relaxed selective constraints in homostylous species might also explain the counterintuitive results of posterior estimates of some traits (e.g., calyx length and corolla-tube length) being overall larger in homostylous than heterostylous species (Figs 3, 4), even though their selective optima (θ) are slightly smaller in homostylous species (Table 2), provided that the posterior mean in the MCMCglmm analysis is not more strongly affected by outliers than θ in evolutionary models (which would make it a currently unknown methodological artifact). If, upon the evolution of homostyly, the optimal trait value shifts to a (slightly) smaller value, congruent with a shifted resource-allocation optimum (Sicard and Lenhard 2011), but the stochastic fluctuations increase so much that some species evolve very large trait values (as for instance evidenced by the triangles in the bottom left quadrant of Fig. 2), then the net effect of homostyly, as measured by the MCMCglmm analysis (Fig. 3), would be shifted toward increased trait size.

To summarize, the release of evolutionary constraints on flowers after the loss of heterostyly (exemplified by higher levels of σ^2 for most traits in homostylous species), combined with the lack of strong selective pull towards new trait optima (exemplified by similar values for α in homostylous and heterostylous species or a single, global α; Table 2), are likely to profoundly affect the trajectory of floral evolution in homostylous primroses. This conclusion contrasts with the theoretical predictions of Glémin and Ronfort (2012), who argued that directional selection toward a new optimum of resource allocation within flowers would be required to explain evolutionary trajectories upon transitions toward high selfing. Extreme empirical cases of a strong selfing syndrome evolving over short evolutionary timespans, such as in the well-studied systems *Capsella rubella* and *Leavenworthia alabamica*, may indeed be triggered by strong positive selection (Foxe et al. 2009; Guo et al. 2009; Busch et al. 2011; Sicard et al. 2011; Slotte et al. 2012), but our results suggest that the evolutionary fate of homostylous primroses is much more variable due to either an increase in the strength of genetic and macro-evolutionary drift and/or a release of selective constraints from pollinators, or alternatively by the adoption of several, distinct evolutionary regimes among homostylous species. To put it simply: while some species evolve a typical selfing syndrome after the transition to homostyly (e.g., *P. cicutariifolia, P. prenantha, P. muscoides, P. watsonii*), others do not (e.g., *P. halleri, P. japonica, P. mollis*; Fig. 1).

Concluding, our findings imply that the reduction of floral size in partially or mostly selfing species is not a general law. Rather, it represents one of several possible outcomes of the loss of self-incompatibility. This view is congruent with Ernst's (1962, p.94) characterization of the transitions in morphological and reproductive characters associated with the loss of heterostyly as "an overall picture of surprising diversity of floral plasticity" (translated from German by JdV), while summarizing his forty-five years of work on the breeding systems of *Primula*. Though data is currently lacking, it would be useful to determine whether pollen/ovule ratios display a similar variability in homostylous species, as lower pollen/ovule ratios are typically considered part of the selfing syndrome (Ornduff 1969; Cruden 1977; Ritland and Ritland 1989). It would also be interesting to investigate why the loss of self-compatibility is sometimes associated with the evolution of smaller flowers and sometimes with the evolution of larger flowers. Does this variation reflect contrasting outcomes of truly neutral genetic drift, or do selective regimes differ among species? Compelling evidence for either scenario will probably stem from a combination of new comparative methods with targeted experiments on reproductive ecology.

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Supplementary information, Table S1. Model fit and estimated parameters of all models of quantitative-trait evolution for the four floral traits, indicating the fit of the model to the data (corrected AIC score, AICc), probability that the model is the best model among the candidate models (AICc weight), and estimated values of the parameters θ (theta; optimum), α (alpha, selective pull) and σ^2 (sigma^2; rate of random drift). When models contain a single, global parameter, estimates are italicized and printed in the center of the column; estimates for heterostyly- and homostyly-specific parameters are reported in their respective columns. NA: parameter not present in model. Results based on stochastic maps for the evolution of heterostyly generated under ARD-model (first page) and SYM-model (second page).

Trait	Model	AICc	AICc weight	*Theta*		*Sigma²*		*Alpha*	
				Heterostyly-specific	Homostyly-specific	Heterostyly-specific	Homostyly-specific	Heterostyly-specific	Homostyly-specific
Character mapping using ARD-model									
Calyx length	BM_1	44.266±0.000	0.000±0.000	*0.751±0.000*		*0.433±0.000*		NA	
	BM_S	45.511±0.146	0.000±0.000	*0.743±0.001*		0.023±0.000	0.025±0.001	NA	
	OU_1	12.642±0.000	0.197±0.007	*0.684±0.000*		*0.958±0.000*		*6.351±0.000*	
	OU_M	11.446±0.039	0.354±0.011	0.623±0.008	0.615±0.008	*0.046±0.000*		*0.309±0.000*	
	OU_{MA}	12.724±0.143	0.183±0.009	0.616±0.008	0.617±0.009	*0.046±0.000*		0.314±0.002	0.316±0.001
	OU_{MV}	12.070±0.266	0.267±0.021	0.620±0.008	0.613±0.008	0.049±0.001	0.055±0.002	0.306±0.001	
Corolla-limb diameter	BM_1	246.270±0.000	0.000±0.000	*1.255±0.000*		*2.153±0.000*		NA	
	BM_S	246.102±0.186	0.000±0.000	*1.246±0.001*		0.127±0.004	0.125±0.004	NA	
	OU_1	173.267±0.000	0.234±0.005	*1.278±0.000*		*60.242±0.000*		*136.543±0.000*	
	OU_M	173.256±0.003	0.235±0.005	1.281±0.001	1.281±0.001	*2.869±0.014*		*6.503±0.031*	
	OU_{MA}	175.753±0.254	0.110±0.010	1.281±0.004	1.284±0.004	*0.332±0.006*		0.631±0.014	0.632±0.014
	OU_{MV}	172.070±0.071	0.421±0.008	1.283±0.001	1.282±0.001	2.780±0.155	2.757±0.125	*4.802±0.192*	
Herkogamy	BM_1	26.371±0.000	0.000±0.000	*0.510±0.000*		*0.376±0.000*		NA	
	BM_S	20.299±0.467	0.000±0.000	*0.521±0.001*		0.030±0.002	0.029±0.002	NA	
	OU_1	-15.705±0.000	0.000±0.000	*0.481±0.000*		*0.891±0.000*		*7.726±0.000*	
	OU_M	-63.575±0.160	0.013±0.011	0.303±0.023	0.298±0.023	*0.140.0.006*		*2.049±0.087*	
	OU_{MA}	-69.210±1.553	0.035±0.009	0.280.0.025	0.319±0.026	*0.090.0.003*		1.847±0.071	1.851±0.071
	OU_{MV}	-83.408±0.427	0.953±0.014	0.313±0.024	0.290.0.024	0.238±0.030	0.186±0.024	*5.073±0.351*	
Corolla-tube length	BM_1	238.612±0.000	0.000±0.000	*1.662±0.000*		*2.026±0.000*		NA	
	BM_S	232.143±0.191	0.000±0.000	*1.613±0.004*		0.136±0.006	0.143±0.006	NA	
	OU_1	202.254±0.000	0.029±0.002	*1.517±0.000*		*4.249±0.000*		*6.228±0.000*	
	OU_M	201.435±0.057	0.048±0.004	1.411±0.015	1.406±0.015	*0.206±0.000*		*0.304±0.000*	
	OU_{MA}	202.261±1.186	0.256±0.016	1.431±0.017	1.416±0.016	*0.184±0.004*		0.233±0.010	0.234±0.010
	OU_{MV}	195.481±0.198	0.666±0.018	1.398±0.014	1.386±0.015	0.284±0.014	0.293±0.014	*0.282±0.001*	
Trait	Model	AICc	AICc weight	*Theta*		*Sigma²*		*Alpha*	

Supplementary information, Table S1, continued.

Trait	Model	AICc	AICc weight	*Theta*		*Sigma²*		*Alpha*	
				Heterostyly-specific	Homostyly-specific	Heterostyly-specific	Homostyly-specific	Heterostyly-specific	Homostyly-specific
Character mapping using SYM-model									
Calyx length	BM_1	44.266±0.000	0.000±0.000	0.751±0.000		0.433±0.000		NA	
	BM_S	43.936±0.063	0.000±0.000	0.75.001		0.02.000	0.04.001	NA	
	OU_1	12.642±0.000	0.247±0.006	0.684±0.000		0.958±0.000		6.351±0.000	
	OU_M	11.578±0.056	0.424±0.005	0.686±0.003	0.525±0.005	0.047±0.000		0.313±0.000	
	OU_{MA}	14.138±0.478	0.157±0.003	0.692±0.002	0.521±0.005	0.045±0.000		0.301±0.005	0.305±0.005
	OU_{MV}	13.427±0.091	0.172±0.007	0.686±0.004	0.522±0.005	0.046±0.001	0.055±0.001	0.308±0.001	
Corolla-limb diameter	BM_1	246.27±0.000	0.000±0.000	1.255±0.000		2.153±0.000		NA	
	BM_S	242.205±0.323	0.000±0.000	1.252±0.001		0.097±0.004	0.351±0.010	NA	
	OU_1	173.267±0.000	0.269±0.005	1.278±0.000		60.242±0.000		136.543±0.000	
	OU_M	173.265±0.000	0.270±0.005	1.277±0.000	1.281±0.000	2.944±0.002		6.674±0.004	
	OU_{MA}	189.454±2.787	0.068±0.005	1.276±0.001	1.131±0.021	0.359±0.019		0.787±0.053	0.809±0.053
	OU_{MV}	172.511±0.080	0.393±0.008	1.278±0.000	1.274±0.002	2.041±0.073	3.692±0.126	4.964±0.164	
Herkogamy	BM_1	26.371±0.000	0.000±0.000	0.51.000		0.376±0.000		NA	
	BM_S	10.642±0.620	0.000±0.000	0.535±0.001		0.016±0.002	0.171±0.007	NA	
	OU_1	-15.705±0.000	0.000±0.000	0.481±0.000		0.891±0.000		7.726±0.000	
	OU_M	-63.019±0.145	0.000±0.000	0.515±0.008	0.068±0.008	0.163±0.007		2.38±0.106	
	OU_{MA}	-64.754±1.280	0.004±0.001	0.512±0.011	0.076±0.014	0.144±0.006		1.921±0.088	1.887±0.087
	OU_{MV}	-81.731±0.201	0.996±0.001	0.502±0.013	0.10.013	0.564±0.036	0.088±0.023	7.845±0.428	
Corolla-tube length	BM_1	238.612±0.000	0.000±0.000	1.662±0.000		2.026±0.000		NA	
	BM_S	222.382±0.191	0.000±0.000	1.643±0.002		0.082±0.004	0.395±0.007	NA	
	OU_1	202.254±0.000	0.005±0.001	1.517±0.000		4.249±0.000		6.228±0.000	
	OU_M	201.719±0.047	0.008±0.001	1.519±0.005	1.301±0.014	0.208±0.000		0.307±0.000	
	OU_{MA}	201.951±1.555	0.321±0.025	1.566±0.008	1.333±0.009	0.127±0.004		0.179±0.014	0.219±0.013
	OU_{MV}	191.999±0.162	0.666±0.025	1.52.006	1.28.009	0.163±0.008	0.604±0.010	0.268±0.000	

Results reported as mean ± standard error across 100 stochastic maps.

APPENDIX V PHYLOGENETIC PATTERNS OF CLIMATIC, HABITAT AND TROPHIC NICHES IN A EUROPEAN AVIAN ASSEMBLAGE

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Aim *The origins of patterns of ecological diversity in continental species assemblages have intrigued biogeographers. We apply phylogenetic comparative analyses to disentangle evolutionary patterns of species ecological niches in an assemblage of European birds. We compare phylogenetic patterns in trophic, habitat and climatic niche components and suggest explanatory hypotheses for diversity in the ecological niches of European birds.*

Location *Europe*

Methods *We identify characteristics of the climatic, habitat and trophic niche of 409 species of breeding birds in Europe. We then apply ordination to reduce niche dimensionality to a number of axes sufficient to explain 90% of the variability of each niche component. We calculate multivariate disparity through time for each component across 100 fully resolved maximum likelihood phylogenetic trees. We compare empirical levels of disparity and phylogenetic signal to expectations based on neutral (Brownian) models of character evolution.*

Results *In this assemblage, related species are generally more divergent in their climatic and habitat niches than they are in their trophic niche. Levels of multivariate disparity are greatest for the climatic niche, followed by disparity of the habitat and the trophic niches. Levels of phylogenetic signal occur in reverse order among these niche components. Disparity markedly exceeds levels*

expected from a neutral model of ecological diversification, thus providing no evidence for phylogenetic niche conservatism in these multivariate niches.

Main conclusions *Phylogenetic patterns in the three niche components differ within this avian assemblage and stimulate new, testable hypotheses on the relative lability of niche components. Variation in evolutionary rates and/or non-random, macroecological sampling likely lead here to differences in the phylogenetic structure of niche components. Testing these hypotheses depends on more-complete phylogenetic trees of the birds, generation of random samples from those phylogenies, and extended ecological data on additional species that are less well-studied than those in Europe.*

Introduction

Macroecologists and biogeographers are asked to identify the phylogenetic characteristics that underlie the impressive ecological diversity of large continental assemblages of animals and plants (Wiens & Donoghue, 2004). This is because phylogenetic analysis of large assemblages can illuminate a variety of new and existing hypotheses for the mechanisms that underlie ecological patterns. For example, absence of phylogenetic signal (i.e. the correlation between species trait similarity and phylogenetic relatedness) in patterns of distribution and abundance of forest birds, along with additional ecological information, can suggest the presence of yet unobserved causative agents that impact native forest bird distribution (Ricklefs, 2011). Similarly, the absence of phylogenetic signal in the climatic niche of European mammals suggests that competition or human influence could play roles in determining the distributions of these species (Dormann et al., 2010). In contrast, phylogenetic signal in plant species that successfully invade a continent might point to shared ecological traits that promote invasiveness (Cadotte et al., 2009). These studies show that analysis of the phylogenetic structure of assemblages can help to identify testable ecological hypotheses. However, exclusive focus on detection of phylogenetic signal limits the scope of questions and hypotheses that macroecological studies can generate by applying phylogenetic methods. The application of more-advanced phylogenetic approaches may reveal previously undetected patterns in continental assemblages, which in turn could suggest more refined hypotheses and a greater variety of research directions.

Multiple approaches have been used for phylogenetic analyses of large species assemblages. One approach quantifies phylogenetic and ecological components of a trait, then determines the geographical distribution of this variation in relation to environment, potentially in the context of a biogeographical hypothesis (e.g. in the study of Bergmann's rule, Olalla-Tarraga et al., 2010). Another approach applies a neutral (Brownian) model of evolutionary diversification to estimate evolutionary rates (Felsenstein, 1985). This has been used to identify phyletic and functional correlates of environmental niche diversity in east Asian grasses (Liu et al., 2011). An alternative to model-based approaches for the estimation of rates of ecological diversification is analysis of trait disparity (Foote, 1993), as has been used to study morphological diversification (Foote, 1997). Calculation of disparity involves the determination of average dissimilarity among species with regard to a set of traits and the apportionment of this variation among and within phyletic groups of species (i.e., clades; Fig.

1). The visualization of disparity through time (DTT) can clarify the temporal development of morphological correlates of ecological diversity (Harmon et al., 2003). A DTT analysis could also be used to identify historical trends in ecological diversification of assemblages, and to compare diversification among types of ecological traits, such as distinct components of the environmental niche.

In this paper, we apply DTT analysis to describe the phylogenetic structure of ecological variability in the continental assemblage of European birds, with the goal of generating explanatory macroecological hypotheses that are potentially testable. This well-studied vertebrate assemblage is an excellent focus for identifying phylogenetic patterns of ecological variation. First, the ample ecological variation of these birds has provided a basis for earlier analysis of phylogenetic characteristics of ecological traits (Brandle et al., 2002; Bohning-Gaese et al., 2003). Second, the ecological variability of birds can be categorized into three distinct components of the species environmental niche (Hutchinson, 1957), for each of which there are abundant data. The habitat niche reflects the preferred habitat use of a species during activities such as foraging and nesting (Grinnell, 1917). In contrast, the trophic niche (Elton, 1927) can be quantified for each species by considering the types of food items taken, the behaviors used in food acquisition, and the substrates from which items are acquired. This niche reflects the food resources preferred by a species and aspects of how a species impacts those resources (Chase & Leibold, 2003). A third niche component is the climatic niche, which reflects the climatic tolerances or preferences of species and can be quantified by relating the occurrence of species to the climate of the occupied geographic area (Austin et al., 1990). These three niche components represent aspects of Hutchinson's realized environmental niche (Hutchinson, 1978), which incorporates the physiological capabilities of species, the habitats available to them, and the restricting effects of ecological interactions.

We calculate indices of phylogenetic signal and develop DTT analysis to describe phylogenetic assemblage structure. We first quantify the ecological characteristics of each species in terms of the three niche components. We use Euclidian distance matrices to describe species separation in a multivariate ecological space for each niche component and use the matrices to calculate multivariate disparity across a sample of fully-resolved molecular phylogenetic trees for the species relationships. We compare estimates of phylogenetic signal for each of the niche components to the DTT results. We also apply DTT to a neutral (Brownian) model of ecological niche diversification. This provides a reference for levels of ecological disparity that would be expected were niches of these particular

species to have diversified in a phylogenetically conserved fashion (Wiens et al., 2010). We then use the indices and DTT analysis to identify differences in phylogenetic structure among the three niche components of the species. When niche diversification within clades has continued over time, disparity values remain high (near unity) over the time spanned by the phylogeny of the species. Differences in indices of phylogenetic signal and DTT among niche components motivate the proposition of testable hypotheses regarding the ecological and geographic processes that are potentially responsible.

Methods

We selected all breeding bird species that were represented in the European Bird Census Council (EBCC) Atlas of European Breeding Birds (Hagemeijer & Blair, 1997), a total of 409 species. These data represent the best available information on the breeding distribution and ecology of this avian assemblage at a 50kmx50km grid resolution. We focused on "The Complete Birds of the Western Palearctic" CD-ROM (Perrins & Ogilvie, 1998) as the data source for the habitat and trophic niches of these avian species because of the extensive treatment and literature review of habitat use, foraging behavior, foraging location, food items, and nesting characteristics. We excluded from consideration all species that feed exclusively in the pelagic zone during the breeding season because data are lacking to meaningfully distinguish trophic differences among species.

We decomposed patterns of diversification for all niche axes of each niche component (climatic, habitat and trophic) separately. We applied multivariate analyses to continuous and discrete ecological characters to produce species-specific values on independent niche axes. We chose to include a number of axes from each niche ordination sufficient to describe a uniform percentage of the total ecological variation (90%) in each niche. The number of axes thus varied among niches, but we needed to assure that any differences among niches during phylogenetic comparative analysis were not due to consideration of arbitrary and variable percentages of ecological variation among niches. Use of a standard proportion of ecological variation for each set of variables enabled us to make consistent and comparable estimates of variability for analysis of ecological divergence of species in terms of the three niche components. All ecological ordinations were conducted in R (R Development Core Team, 2011) with the package ade4.

Trophic Niche

The 35 variables of the trophic niche (Elton, 1927) were non-exclusive, focused on the breeding season, and included variables that characterized food type (14 variables), behaviors used in acquiring food (9), substrate from which food is taken (9), and the period of day during which a species forages actively (3). The variables were scored as either 0 or 1, with the exception of body weight, which was scored as an average of values provided for individuals weighed during the breeding season (Table 1). Body weight was included as a trophic variable because body weight is likely correlated with total energy needs and prey size (Price et al., 2000). A food type variable (e.g., small birds, seeds, invertebrates, etc.) was scored as 1 when the substance reportedly formed more than 10% of stomach contents, 10% or more of observed foraging successes, or when the species was described as principally foraging on a particular item based on qualitative observation. Items described exclusively as being taken only "rarely", "occasionally", or presented as a unique observation for a species, led to a score of zero (0) being assigned to the type of food item under consideration.

Species received a score of one (1) on each foraging behavior attributed to the species, unless the species was described as "rarely" performing a behavior. The nine substrate variables received a score of one (1) if specified in description of foraging behaviors for species. Three variables (nocturnal, crepuscular, and diurnal) described the daily period of foraging activity and were scored as one (1) if the species was reported as being active (or "occasionally active") during that period. When information existed regarding variation in trophic variables between the breeding and non-breeding seasons, only data concerning the breeding season (e.g., food type) were scored as being used. We scored behaviors and food types as unused (0) when they were reported for the species but exclusively from a geographical area outside Europe. Body weight was centered and normalized to unit standard deviation before ordination. Hill-Smith ordination (Hill & Smith, 1976) was used to summarize the matrix of binary and mixed variables.

Habitat Niche

A total of 38 habitat variables (Table 1) were distinguished from climatic variables because the former are central to the original definition of the habitat niche (Grinnell, 1917). We developed 18 variables to describe habitat preferred for nesting (breeding habitat niche). Three mutually exclusive variables were used to describe nest position (elevated > 1m in tree or bush; tree-hole; ground or other substrate or surface). Other nesting habitat categories

were not mutually exclusive and a score of 1 was assigned to additional breeding habitat variables to describe the nesting preferences of the species. An additional 20 variables described the habitat used for foraging (the foraging habitat niche). In both types of habitat niche, all types of wet grassland (wet tundra, fen, sedge meadow, seasonally-flooded meadow, etc.) were pooled. Dry grassland, steppe, and agricultural fields were pooled and shrub and bush were also pooled. These grassland variables were presented as both foraging and breeding habitats.

Sand, beach, and gravel were also pooled into one category that was present as both breeding and foraging niche variables. Mud flats and wind or water-deposited silt habitat were pooled but only considered foraging habitat. Near-shore marine habitat was a foraging variable that was scored for species feeding in marine waters, within 500 m of shore during the breeding season. Forest edge was scored when the species description specified that this habitat was used for foraging. We pooled naturally open forest, open forest caused by disturbance, and early stage successional forest, and included the category in both the breeding and foraging habitat niches. We distinguished garden habitats and urban environment to accommodate European species with populations that breed and/or forage in one or both of these anthropogenic habitat types. Variation in habitat niche among species was quantified using multiple correspondence analysis (Tenenhaus & Young, 1985).

Climatic Niche

We estimated the breeding distribution of each species by determining both confirmed and suspected breeding from digital maps (Hagemeijer & Blair, 1997). Data to describe the climatic niche of species was based on data of species distributions from the EBCC atlas at 50x50 km resolution and nineteen bioclimatic variables at the same resolution (Table 1) from the WorldClim database (Hijmans et al., 2005; www.worldclim.com). We estimated species climatic niches (Austin et al., 1990), using the Outlying Mean Index analysis (OMI, Dolédec et al., 2000). Climatic niche position for each species along the two selected axes was extracted and used as a measure of climate preference (Thuiller et al., 2004).

Phylogenetic Data

We constructed phylogenetic trees for the European birds (Thuiller et al., 2011) with DNA data available in GenBank: 10 mitochondrial gene regions (12S, ATP6, ATP8, COII, COIII, ND1, ND3, ND4, ND5, ND6) plus 6 nuclear regions (28S, c-mos, c-myc, RAG1, RAG2, ZENK). The sequences were aligned with several algorithns and checked by eye. The best

alignment for each region was selected with MUMSA (Lassmann & Sonnhammer, 2006) and depurated with TrimAl (Capella-Gutierrez et al., 2009). Then, DNA matrices were concatenated to obtain a supermatrix. We conducted phylogenetic analyses using Maximum Likelihood within RaxML (Stamatakis et al., 2008) and applied a prior tree constraint at the ordinal level for the birds dataset (based on Hackett et al., 2008). We kept 100 most-likely trees for our analysis to account for the uncertainty associated with phylogenetic inference. The best ML tree can be found in Treebase (http://treebase.org/treebase-web/home.html; study number 10770).

Phylogenetic Signal of Niche Traits

We evaluated for each niche component the degree to which evolutionary relatedness of species was correlated to ecological similarity (i.e. shows phylogenetic signal) by examining values of Pagel's λ and Kappa (Pagel, 1997). These metrics are relatively insensitive to tree size and tree uncertainty (Münkemüller et al., 2012). We calculated Pagel's λ with the R package phylosig and Kappa with pgls in the R package caper. We tested the significance of λ and Kappa with a likelihood ratio test. To make sure our evaluation of phylogenetic signal was independent of the Pagel statistics, we also examined values of Blomberg's K (Blomberg et al., 2003), another frequently-used metric, which we calculated using pgls in the R package caper. We determined the significance of Blomberg's K with a 999x randomization of tip values. We calculated these indices by using the species values on each of the ordination axes that were selected from analyses of niches. We repeated the calculations across 100 nearly-maximum likelihood trees to account for topological uncertainty in the species phylogeny. We compared values of the indices among niche components using Kruskal-Wallace and Wilcoxon tests. We made these comparisons on the maximum likelihood tree only because the other 99 trees were not necessarily independent. The results of the λ and Kappa statistics were nearly identical so we report only the results for λ in addition to results for Blomberg's K.

Disparity Through Time

We began the DTT analysis by calculating for each niche component the Euclidian ecological distance among all pairs of species in the multivariate space defined by species scores on the selected axes of the three ordinations. We then used these distance matrices to compute the mean disparity of all extant subclades at each time step (marked by node ages) across a tree. We then plotted the mean disparity values of all subclades against the age of the parent node

subtending these subclades (Harmon et al., 2003). We accounted for phylogenetic uncertainty by conducting these calculations on 100 most-likely phylogenetic trees. We also performed 1000 multivariate Brownian simulations on each niche and tree combination. These calculations used the inferred variance-covariance matrices of each niche component (Revell & Collar, 2009). We calculated DTT identically for each simulation and plotted for each niche and time step the interval that spanned 95% of the values. The comparisons of empirical and modeled disparity are robust to incomplete taxon sampling that generally occurs on assemblage trees because empirical disparity curves were compared to corresponding neutral (Brownian) expectations based on phylogenetic species divergences.

Results

The first 22 of 35 axes of the Hill-Smith ordination of the trophic niche capture 90% of trophic niche differentiation among all species. The first 24 of 38 axes of the multiple correspondence analysis of the habitat niche captured 90% of differentiation among all species. The first two of 19 axes of the OMI analysis of climatic niche values captured 90% of climatic niche differentiation between species. A high proportion of nodes of the maximum likelihood phylogenetic tree were supported by RaxML bootstrap analyses (BS): 71.1 % of the nodes had BS equal or higher than 70%; 8.2% of the nodes received low support (BS of 50-70%); and 20.8% of the nodes received no support (BS < 50 %).

Phylogenetic Signal

The analysis of phylogenetic signal shows that significant, non-zero values of λ, and K appear in some elements of the habitat and trophic niches (Figs. 2, 3). However, values of these indices are generally low across all three niches (Fig. 3). The percentage of axes-by-phylogenetic tree combinations with significant phylogenetic signal is highest for the trophic niche and lowest for the climatic niche. Kruskal-Wallis tests reveal significant differences exist among niches for both λ (K-W X2 = 17.2, df=2, P<0.001) and K (K-W X2=6.7, df=2, P=0.036). For λ, values for trophic niche differ from those of both the climatic (Wilcoxon W=1, P=0.015) and habitat (W=440, P<0.001) niches. For K, values for the climatic niche are lowest and differ significantly from those of the habitat niche (W=3, P=0.037) while other comparisons are non-significant. These trends demonstrate that related species of European birds resemble one another less in the climates they prefer during breeding, and in the habitats they use, than in their food preferences.

Disparity Through Time Analysis

Multivariate disparity of the climatic niche of European breeding birds exceeds that of the other two niche components (Fig. 4). The nearly constant level of disparity in climatic niche among the 100 trees indicates that over the evolutionary history of this assemblage there is more variation within related clades than would be expected under a neutral (Brownian) evolutionary model of ecological divergence (Fig. 4). Similarly, disparity values for the habitat niche are consistently greater than expected under the Brownian model, beginning very early on the trees (Fig. 4). Further, the disparity of the trophic niche is similar to the disparity for the other two niche components, but of lower magnitude.

Discussion

Values of λ and K indicate that European birds appear more divergent than is suggested by a model of neutral evolutionary divergence, in regard to all three components of the ecological niche. Consistent with the values of these indices, the pattern of disparity for each of the niche components exceeds expectations if species niches were to diverge according to a neutral (Brownian) model. These indices and the disparity values for the three niche components suggest that there is greater phylogenetic structure in the trophic characteristics of the species in this assemblage than in the other two niche components. This trend appeared early in the evolutionary history of these species and has been maintained during the last 20 million years, since the early Miocene. The mechanisms that could potentially contribute to this pattern may be related to sampling lineages or species from an underlying phylogeny or, alternatively, to rates of niche diversification that have affected these species. Several hypotheses on the identity of these mechanisms exist and find variable levels of support.

Niche Disparity, Conservatism, and Species Sampling

One hypothesis for niche variability in this assemblage suggests that the ecological similarity of species should be correlated with their degree of phylogenetic relatedness, because of the tendency of species to retain ancestral characteristics (Wiens et al., 2010). Our representation of conserved trait evolution through the implementation of a neutral model for niche diversification provided expected levels of disparity that would arise in conservatively evolving ecological traits (Wiens et al., 2010). In contrast, the empirical patterns we observe suggest that the evolutionary history of the three niches, as represented by this assemblage,

exhibit enhanced levels of disparity compared to the conservative expectation. Levels of disparity also differ among the niche components, a pattern with at least two potential origins.

Observed differences in disparity among niche components could arise due to a non-random sampling process ("macroecological sampling") that operates across the clade of all birds and results in the current composition of the European assemblage. This could occur in the absence of differences among niche components in their evolutionary rates. A macroecological sampling bias would arise when the birds of the European assemblage are not a random sample from the inclusive clade of birds of which they are members. This sampling bias would clearly not be anthropogenic in origin. Observed differences in DTT among niches could also involve heterogeneity in rates of niche evolution within the complete clade from which the European avifauna is drawn (Diniz-Filho et al., 2010). Such non-stationarity in rates of niche diversification, in combination with incomplete and biased taxon sampling from within the encompassing clade, might create the appearance of heterogeneity among niche components in their DTT values. However, this heterogeneity might not represent well any rate heterogeneity in the full tree of birds due to the sampling bias.

Macroecological sampling of the lineages in this assemblage could involve additional evolutionary processes that are not identified as niche evolution per se. These might include range evolution during vicariance events and the development of migratory behavior (Lundberg, 1988; Perez-Tris et al., 2004; Mila et al., 2006), which likely involves both environmental factors and factors intrinsic to populations. One result of migratory behavior on niche characteristics is that the environmental niche of migratory birds during the breeding season may be less conserved than in the winter range (Martinez-Meyer et al., 2004). This suggests that species niche characteristics during residency in the breeding range may become labile during the evolution of migratory behavior. Change in migration could also contribute to geographic variation within species in their niche characteristics (Martinez-Meyer et al., 2004), as in the case of partial migration (Lundberg, 1988), and could also be a proximal mechanism by which macroecological sampling occurs.

Hypotheses Regarding Evolutionary Rates

Four potentially conflicting hypotheses attribute the primary origin of avian ecological diversity (a) to rates of divergence of the climatic niche arising from global climate cycling and geographical barriers (Lovette, 2005; Outlaw & Voelker, 2008), (b) to rates of divergence of habitat use (Lack, 1944; Richman & Price, 1992; Cicero & Johnson, 1998), (c) to rates of

divergence of trophic preferences (Richman & Price, 1992; Grant & Grant, 2006), or (d) to rates that are consistent with neutral evolutionary drift (Felsenstein, 1985; Wiens et al., 2010). Our results firmly suggest that neutral (Brownian) evolution (d) alone is not adequate to explain the variety of among-niche patterns of disparity we observe. However, each of the other hypotheses has some support in the literature and differences in rates of diversification of the three niche components could exist, thus necessitating a set of hypotheses as an alternative to macroecological sampling bias.

Ecological diversification of the climatic niche as shown in DTT is notably greater than of the other two niches. This suggests the hypothesis that the primary evolutionary driver of ecological diversity held in these birds arises from higher rates of diversification of the climatic niche. One potential mechanism for this involves global climate cycling (Lovette, 2005). Global climate variability has repeatedly driven species in and out of geographically isolated refugia in the northern hemisphere to promote vicariant speciation (Lovette, 2005), potentially since the Pliocene (Guillaumet et al., 2008; Outlaw & Voelker, 2008). Nonetheless, ecological diversity in European birds is the product of ecological diversification in many different clades, many members of which are not present in Europe. Knowledge of these species would be necessary for unbiased rate estimation. Relatively rapid diversification of the climatic niche, if confirmed, would potentially contrast with rates of niche evolution in tropical avian assemblages (Peterson et al., 1999; Wiens & Donoghue, 2004), where effects of climate cycling on niche diversification could be less important (Lovette, 2005).

Divergent preferences for feeding and breeding habitat exist among many closely related avian species (Lack, 1944). Diversification of ecological traits in some avian groups primarily involves diverging habitat preferences (Richman & Price, 1992; Cicero & Johnson, 1998). Our results suggest that evolutionary divergence of habitat preferences could contribute to ecological diversity, second only to diversification of the climatic niche. A test of this hypothesis could be devised based on a random sample of species from a more-complete phylogenetic tree that includes the European avian assemblage. Rates of ecological diversification of climatic and habitat niches of the species in such a sample could be calculated and compared using DTT. A different approach could use phylogenetic regression on the same random sample to examine the statistical effects of migratory status and climatic niche dissimilarity, in order to apportion diversification in habitat preference to these two factors.

The limited diversification of trophic habits could be due to relatively slow evolutionarily rates. For example, constraints might occur by slow or infrequent divergence of specialized morphology that is associated with resource capture and feeding, and which may have diversified relatively early in lineage history (Richman & Price, 1992; Cicero & Johnson, 1998; Bohning-Gaese & Oberrath, 1999). Our results are partially consistent with earlier studies that report evidence for phylogenetic signal in the trophic niche of the assemblage of European birds. Böhning-Gaese and Oberrath (1999) use Mantel tests on an ecological dissimilarity matrix and a matrix representing phylogenetic distance among 151 species to find phylogenetic signal in both diet and breeding habitat. Similarly, Brandle et al. (2002) examines taxonomic levels to find that significant phylogenetic conservatism exists in dietary niche breadth of 142 European species at the level of family and genus. The pattern we observe in the trophic characteristics exhibits weaker phylogenetic signal than these previous studies, perhaps because our extensive sample of trophic traits may be more representative of the ecological divergence of European species. Nevertheless, one potentially testable hypothesis is that low levels of disparity of trophic preferences (compared to the other niches) arise because macroecological sampling preferentially 'selects' lineages having particular trophic characteristics. Such sampling, and not variation in evolutionary rates, could be the cause of this pattern.

Conclusions

Over the history and diversity represented by the European avifauna, we find no evidence for clear phylogenetic conservatism of the three multivariate niche components we investigated. While phylogenetic niche conservatism has been proposed as an evolutionary principle, this perspective may arise from focus on a small number of ecological characteristics or a biased sample thereof. Alternatively, conservatism of some avian niche components may be greater in other regions of the world, some of which (e.g., tropical areas) are much more speciose than Europe and thus may have greater influence on global trends. Several processes may contribute to the patterns we observe here, including the processes of macroecological sampling and relative rates of ecological diversification of niche components. Hypotheses regarding the importance of variation in underlying evolutionary rates and macroevolutionary sampling for variation in DTT and phylogenetic signal may be testable with greater ecological and phylogenetic information. The application of additional and developing methods in comparative analysis to large assemblages will likely reveal new insights that can drive hypothesis generation and testing.

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Tables

Table 1 Original variables used in the analyses on niche diversification. With the exception of body mass, all habitat and trophic niche variables were scored as used (1) or not used (0).

	Habitat		
Climate	**Foraging**	**Nesting**	**Trophic**
Annual Mean Temperature	Wet grassland, fens, tundra	Wet grassland, fens, tundra	Seed, nuts, grain
Mean Diurnal Range	Dry grassland	Dry grassland	Frugivory
Isothermality	Rocky slope	Banks of sand or mud	Vegetative parts
Temperature Seasonality	Fast lotic	Near water, lakeshore, island	Invertebrates
Maximum Temperature of Warmest Month	Still or slow lotic	Sand or gravel beach	Fish
Minimum Temperature of Coldes Month	Near shore marine	Reed marsh	Small mammals
Temperature Annual Range	Salt marsh	Conifer forest	Large mammals
Mean Temperature of Wettest Quarter	Mud or silt flat	Deciduous forest	Herptiles
Mean Temperature of Driest Quarter	Sand or gravel beach	Mixed forest	Vertebrates (unspec.)
Mean Temperature of Warmest Quarter	Reed marsh	Mediterranean or oak	Small birds
Mean Temperature of Coldest Quarter	Conifer forest	Open or low forest	Large birds
Annual Precipitation	Deciduous forest	Shrub, bush, or brush	Large bones
Precipitation in Wettest Month	Mixed forest	Urban	Carrion
Precipitation in Driest Month	Mediterranean or oak	Garden	Pursuit
Precipitation Seasonality	Open or low forest	Rock faces, outcrops, structures	Sally
Precipitation of Driest Quarter	Forest edge	Nest position (elevated, hole, ground)	Foliage glean
Precipitation of Wettest Quarter	Shrub, bush, brush		Pounce
Precipitation of Warmest Quarter	Urban		Graze
Precipitation of Coldest Quarter	Garden		Pick, peck, stab
	High air		Dig
			Overturn objects
			Probe
			Water surface
			Underwater
			Water (unspecified)
			Mud
			Terrestrial ground
			Canopy
			Shrub
			Vegetation (unspec.)
			Air
			Nocturnal
			Crepuscular
			Diurnal
			Body mass

Figures

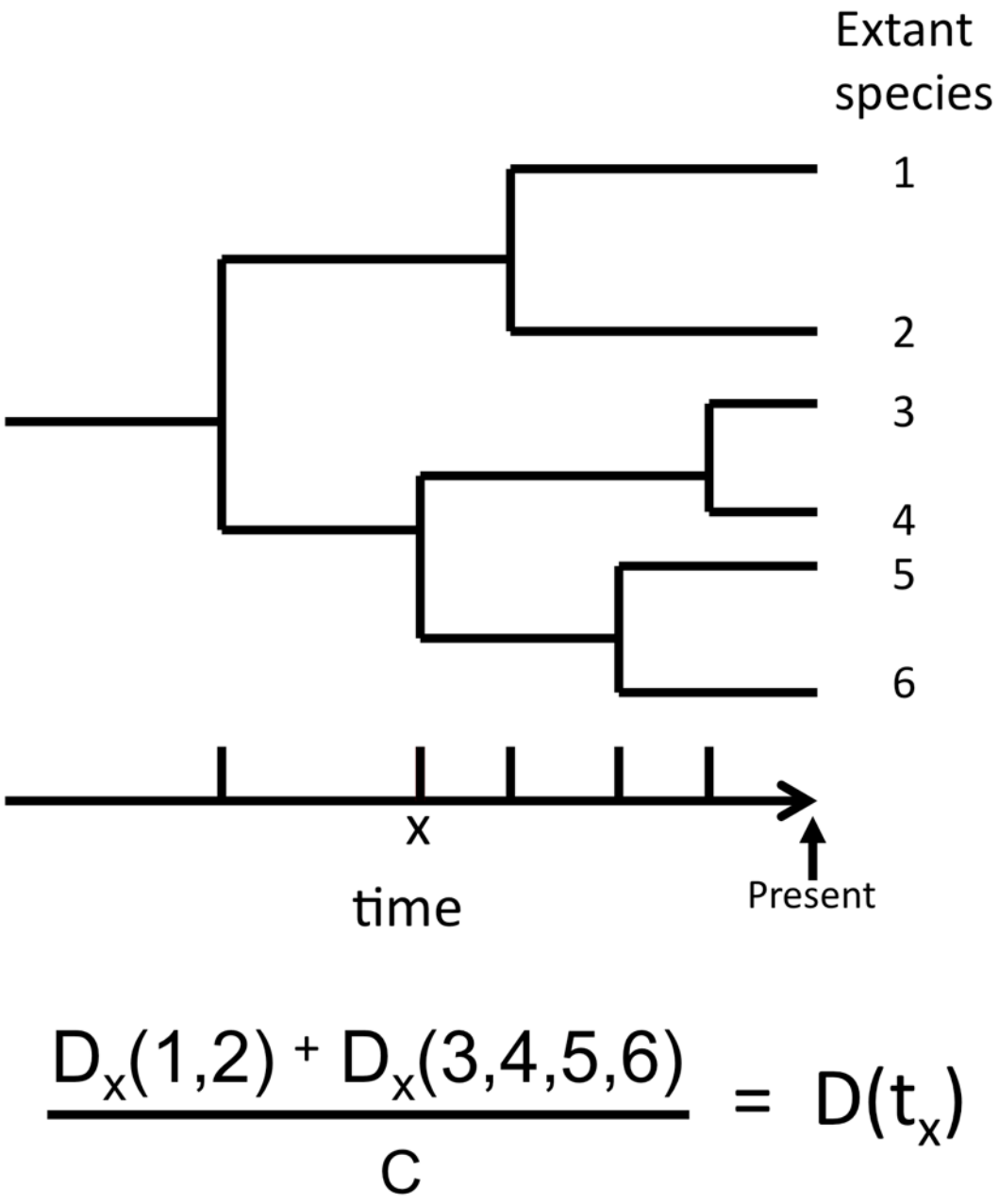

Figure 1 Calculation of disparity for a time point on a hypothetical phylogenetic tree. For a particular time point, corresponding to the date of an interior node, disparity of an ecological trait is calculated for each existing clade (lineage) as the average Euclidian distance among the clade members, in regard to the trait(s) under consideration. In the figure, total disparity at time x, $D(t_x)$, is equal to the sum of the disparity values calculated for each of the two clades existing at time x, D_x, one clade with species 1 and 2 and the other with species 3 through 6, divided by the number of clades, C, which here is two. In a disparity through time analysis, values of disparity are determined for the date of each node along the phylogeny.

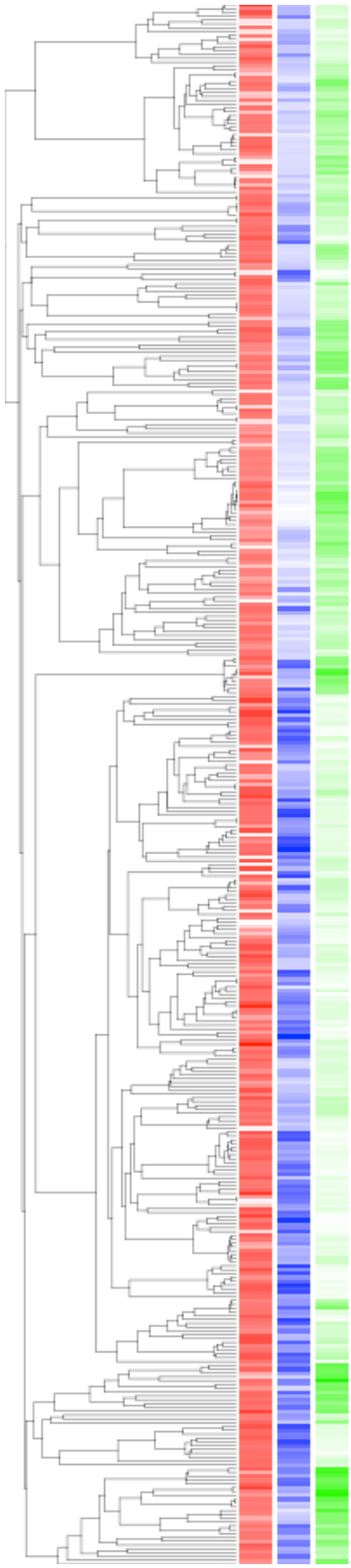

Figure 2 Niche diversification on the maximum likelihood phylogenetic tree among 409 avian species of the western Palearctic. Color swaths represent species scores on the first eigenvector from ordinations of the climatic (red), habitat (blue) and trophic (green) components of the environmental niche. Species scores on each vector have been standardized to range from zero (maximum color intensity) to unity (white), and transformed to approximate a normal distribution. Clustering of color of similar intensity among closely related terminal nodes (species) indicates similar eigenvector scores, and suggests the presence of phylogenetic signal, notably in the trophic niche.

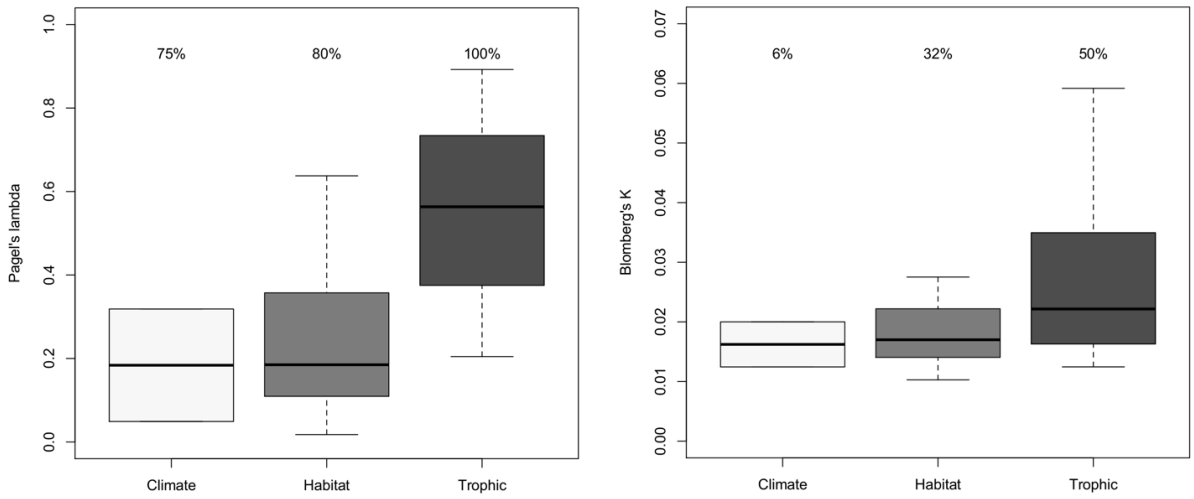

Figure 3 Boxplots of Pagel's λ and Blomberg's K as measures of phylogenetic signal for three components of the Hutchinson's ecological niche. The median, 1st quartiles, and 1.5 times the quartile value are obtained by calculating λ and K statistics on the species scores on each eigenvector of each niche component, which together account for 90% of the variability of each niche component. This is repeated here for 87 of the100 sampled phylogenetic trees for which all statistics converged to a stable value. The median values indicate relative phylogenetic signal. The percentages of all values (numbering 87 trees multiplied by the number of eigenvectors) that are significantly greater than zero (alpha = 0.05) are shown above each bar. Substantial variation around the median values indicates that the magnitude of phylogenetic signal varies among the eigenvectors within niche components and with replicate phylogenetic tree. The values of both statistics indicate low levels of phylogenetic signal for eigenvectors in all three niche-components, in spite of statistical significance.

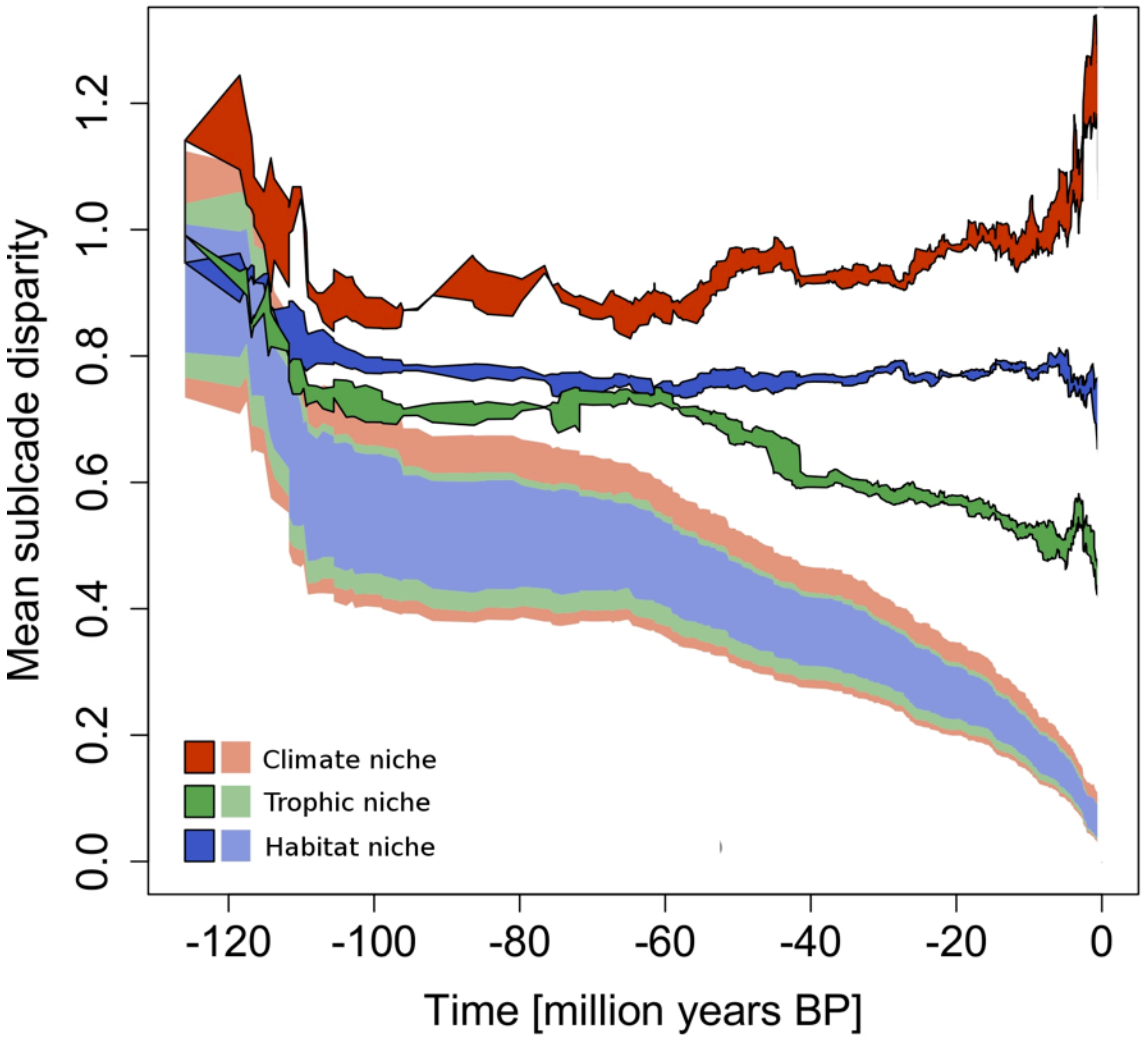

Figure 4 Plots of observed disparity through time (DTT) for observed data and results from Brownian evolution models for three components of the environmental niches of 409 species of European birds. The breadth of solid lines indicates the range of disparity values that arises from topological uncertainty across 100 nearly-maximum likelihood phylogenetic trees. Values of disparity for all three niches (saturated colors) differ from neutral expectations derived from 1000 Brownian evolution simulations of each niche (pale colors).

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